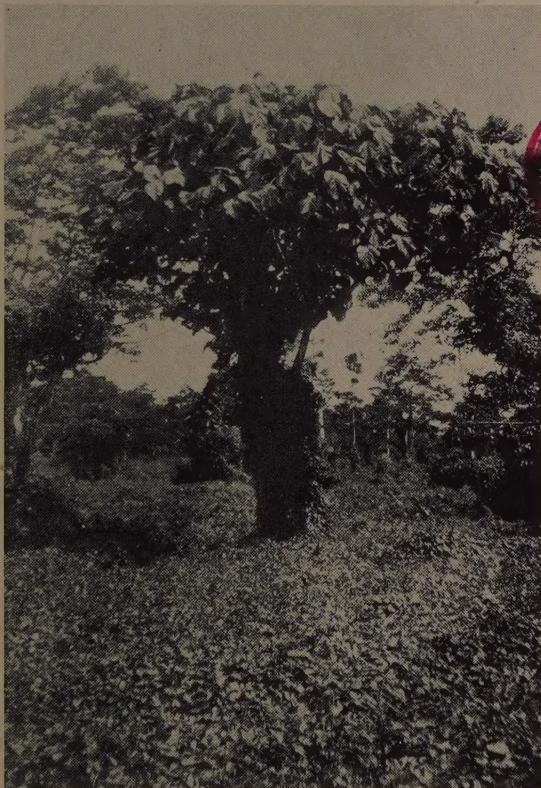


VOLUME XLIV

NUMBER 4

# THE HAWAIIAN PLANTERS' RECORD



The weed pest, *Mikania micrantha*, covering the ground and climbing into a large *lau pata* tree in the lower forest zone behind Safune, Savaii, at an elevation of about 1,700 feet. (Photograph by E. H. Bryan, Jr., courtesy Bernice P. Bishop Museum.)

FOURTH QUARTER 1940

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# THE HAWAIIAN PLANTERS' RECORD

Vol. XLIV

FOURTH QUARTER 1940

No. 4

*A quarterly paper devoted to the sugar interests of Hawaii and issued by the Experiment Station for circulation among the plantations of the Hawaiian Sugar Planters' Association.*

## In This Issue:

### *Nitrogen-Potash-Sunlight Relationships:*

A study of the interrelations of nitrogen, potash, and sunlight reveals that when sunlight conditions are such as to limit the complete assimilation of applied nitrogen and potash fertilizers, sugar yields will be adversely affected.

### *A Devastating Weed:*

In some of the Pacific Islands and in parts of the Asiatic mainland, directly connected with Hawaii by steamer and airplane, there occurs a rampant vine that rates as a pest of prime importance. This vine and its potential danger to Hawaii are described, and a warning is issued against the possibility of its introduction or immigration into the Hawaiian Islands.

### *Colchicine in Relation to Sugar Cane Breeding:*

A new method of producing "improved varieties" of plants (*polyploids*), in which considerable popular interest has been aroused because it is now successfully used with many varieties of plants, promises to become a practical tool in the hands of the geneticist. This method consists simply of applying solutions of the drug, *colchicine*, to growing points, buds, or seeds with the result that plants developing after such treatments may be vegetatively larger in size and more vigorous than the parent plants, as also polyploid hybrids, produced by Mendelian crossing, may be. Flowers and fruit may be larger than normal and sterile hybrids changed into fertile ones.

Colchicine, a highly poisonous alkaloid drug, is obtained from the bulb and seeds of the autumn crocus (*Colchicum autumnale*). In order to understand its effects on plants it is necessary to understand something of *cell division*, *chromosomes*, and *inheritance*, brief explanations of which are given, illustrated by photomicrographs of cells of the sugar cane plant.

Definite effects of colchicine on sugar cane are described and illustrated.

*The Factor of Synergism in Chemical Weed Control:*

Methods of chemical weed control are described which employ modifications of an existing procedure. It is believed that the modification will by-pass the major disadvantages of the older system of control.

The synergistic influence of an added substance to a reduced concentration of an otherwise objectionable herbicide appears to accomplish a satisfactory weeding effect.

A new, mild weed spray solution is described and recommended for field trial on the basis of its almost non-poisonous, non-soil-sterilizing character, reduced cost, high concentration and simplicity of chemical composition, free miscibility with water, and non-corrosive action on rubber and metal parts of spraying equipment.

Several phases of modern weed control are discussed.

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*Further Studies in Nitrogen Nutrition:*

A series of plots was laid out at Makiki to further study the effect of nitrogen fertilization on the growth and yields of the sugar cane plant; to study the physico-chemical and biochemical changes within the cane plant; and to establish a clearer theory of sugar metabolism and storage and its use in the plant as affected by age and season, irrigation and varieties. In this paper a fundamental group of inter-locking findings are discussed regarding the effect of nitrogen when applied in one application. It is to be followed by papers which discuss time of nitrogen application, irrigation, and varieties.

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## Nitrogen-Potash-Sunlight Relationships

By R. J. BORDEN

Information that is concerned with the interrelations of effects produced on sugar cane by sunlight and fertilizers is not abundant and yet it is quite generally agreed that these factors are major influences in sugar cane agriculture. In a previous study (A-105—No. 118)\* we were unable to show any significant interaction between sunlight and nitrogen. We were not entirely satisfied with the results and wished to check them again, with certain changes in our original plan. At the same time we had gathered the idea from certain Rothamsted reports that a relationship had been found between sunlight and potash fertilization. We therefore set up a skirmish test (A-105—No. 118.01) designed with the hope that it would contribute to a better understanding of the interactions which might exist between extra nitrogen and potash fertilization and different sunlight conditions.

The plan used was a factorial design in which three levels of nitrogen and two levels of potash fertilization, with amounts above what were considered average requirements, were studied in all possible combinations with two variations in sunlight conditions. Thus we had 12 combined treatments as follows:

Nitrogen	Potash	Sunlight	Nitrogen	Potash	Sunlight
1. Average	Average	Full	7. Average	Average	Decreased
2. Average	Extra	Full	8. Average	Extra	Decreased
3. High	Average	Full	9. High	Average	Decreased
4. High	Extra	Full	10. High	Extra	Decreased
5. Excessive	Average	Full	11. Excessive	Average	Decreased
6. Excessive	Extra	Full	12. Excessive	Extra	Decreased

Each treatment was installed with 4 replications in Mitscherlich pots that were filled with identical amounts of a well-drained Manoa soil which had been adequately and similarly fertilized with phosphate. The pots were placed on flat cars that could be quickly placed under a glass-roofed shed during rains. Decreased sunlight was provided by lining the tops and sides of one of these glass-roofed sheds with a single thickness of burlap which was quite effective in reducing the amount of direct sunlight.

Two single-eye cuttings of the cane variety 31-1389 were planted in each container on December 29, 1938. Thereafter, during the following five months, all pots received adequate and identical amounts of nitrogen and potash and were grown in full direct sunlight and with all treatments uniform. At the beginning of the sixth month the differentials were imposed. The plants receiving "decreased sunlight" were placed in the shaded house every day at noon, thus they were in

\* Sunlight-Nitrogen relationships, The Hawaiian Planters' Record, 43: 227-235, 1939, by R. J. Borden.

direct sunlight only during the morning hours. The plants receiving "average" nitrogen were thereafter given only  $2\frac{1}{2}$  grams of nitrogen, as compared with 5 and with 10 grams for the "high" and "excessive" nitrogen groups. Similarly, the "average" and "extra" potash groups were subsequently given  $2\frac{1}{2}$  and 10 grams of potash respectively.

The cane was harvested at the age of one year and we have a considerable amount of data. These have been subjected to statistical study by analysis of variance and the significant effects are quite nicely brought out. These may now be discussed.

#### *The Main Effects:*

We have summarized the main effects that were measured from the three separate factors but shall not take much space to discuss them since they quite generally conform to the expected, and our chief interest from this study is in their interactions.

#### *Effects from Nitrogen:*

TABLE I  
(Averages of 16 Pots)

Nitrogen level	Lbs. cane	Purity	Y %	C %	Lbs. sugar	% N in juice	% K <sub>2</sub> O in juice	Total dry wgt. (gms.)
Average .....	4.35	89.0	13.28	.58	.032	.225	951	
High .....	4.58	84.6	11.33	.54	.072	.221	982	
Excessive .....	4.37	79.7	9.62	.44	.189	.204	953	
Difference required for odds of 99 to 1.....	ns	1.6	.54	.04	.010	.014	ns	
(ns = treatment effect is not significant.)								

Apparently our "average" nitrogen level was quite adequate for this 12-month cane crop since there was no reliable gain in yield for the "high" or for the "excessive" N levels. The data clearly show the injurious effects which non-assimilated nitrogen within the plant can have upon the juice quality.

#### *Effects from Potash:*

TABLE II  
(Averages of 24 Pots)

Potash level	Lbs. cane	Purity	Y %	C %	Lbs. sugar	% N in juice	% K <sub>2</sub> O in juice	Total dry wgt. (gms.)
Average .....	4.39	84.0	11.25	.51	.093	.166	961	
Extra .....	4.48	84.9	11.56	.53	.103	.268	963	
Difference required for odds of 19 to 1.....	ns	1.3	ns	.03	.008	.012	ns	
(ns = treatment effect is not significant.)								

As was the case with nitrogen, it is apparent that the potash requirements of the crop were quite adequately satisfied by the "average" application and that the

"extra" potash was without a differential effect upon the cane yields. The difference in the sugar yield is indicative but not highly significant. A better purity is suggested from the "extra" potash level even though the difference is not highly significant, because this treatment has a significantly higher per cent N in the juice and this is quite a contrary relationship to find associated with a better purity in comparisons of this sort.

#### *Effects from Sunlight:*

TABLE III  
(Averages of 24 Pots)

Sunlight condition	Lbs. cane	Purity	Y % C	Lbs. sugar	% N in juice	% K <sub>2</sub> O in juice	Total dry wgt. (gms.)
Full .....	5.04	88.7	13.55	.68	.065	.184	1125
Decreased .....	3.83	80.2	9.27	.36	.131	.250	798
Difference required for odds of 19 to 1.....	.23	1.3	.44	.03	.008	.012	39
(ns = treatment effect is not significant.)							

It is quite clear that the sunlight effects have dominated the results that were obtained. Lower yields, poorer quality, and much higher amounts of non-assimilated nitrogen and potash in the plants are found when sunlight has been a limiting growth factor.

#### *First-Order Interactions:*

Our primary interest in this study was in the relations between sunlight and nitrogen, and sunlight and potash. Since data concerned with the interaction of potash and nitrogen were available we have included it also. Second-order interactions between all three factors were not highly significant so they have not been included in our subsequent discussion.

#### *Interaction between Sunlight and Nitrogen:*

TABLE IV  
(Averages of 8 Pots)

Measurement	Sunlight	Average N	High N	Excessive N	Difference required
Lbs. cane.....	Full .....	4.79	5.26	5.07	.40
	Decreased .....	3.91	3.91	3.68	
Purity .....	Full .....	90.5	89.8	85.8	2.2
	Decreased .....	87.5	79.4	73.7	
Y % C.....	Full .....	14.52	14.07	12.05	.76
	Decreased .....	12.03	8.60	7.18	
Lbs. sugar .....	Full .....	.70	.74	.61	.06
	Decreased .....	.47	.34	.27	
% N in juice.....	Full .....	.024	.046	.125	.015
	Decreased .....	.040	.099	.254	
% K <sub>2</sub> O in juice.....	Full .....	.195	.181	.175	ns
	Decreased .....	.255	.260	.234	
Total dry wgt. (grams).....	Full .....	1074	1155	1147	68
	Decreased .....	829	808	756	

(ns = no significant interaction between sunlight and nitrogen on the per cent K<sub>2</sub>O in the crusher juice.)

These data show us that the differences in sunlight have had some quite different influences upon the effects from the extra nitrogen applications. For instance, under full sunlight conditions, the high nitrogen gave increased cane yields which were of only slightly, if at all, poorer quality than were obtained from the "average" nitrogen treatment, but with decreased sunlight, this high nitrogen not only did not increase the cane yield but it quite definitely produced a cane with poorer quality.

Under decreased sunlight, each successive nitrogen increment has resulted in less sugar, while under full sunlight the sugar yield was adversely affected only by the excessive nitrogen level.

With average nitrogen applications the percentage of nitrogen in the crusher juice was not increased as a result of the reduced light conditions to the same extent as it was when the larger amounts of nitrogen were supplied under a sunlight deficiency.

The suggestion is quite clear that when cane is grown under conditions where sunlight is apt to be deficient, high nitrogen applications must be avoided or maximum sugar yields will not be secured.

#### *Interaction between Sunlight and Potash:*

TABLE V  
(Averages of 12 Pots)

Measurement	Sunlight	Average K <sub>2</sub> O	Extra K <sub>2</sub> O	Difference required
Lbs. cane .....	Full .....	4.96	5.12	ns
	Decreased .....	3.82	3.85	
Purity .....	Full .....	88.6	88.8	ns
	Decreased .....	79.3	81.1	
Y % C.....	Full .....	13.38	13.72	ns
	Decreased .....	9.13	9.41	
Lbs. sugar .....	Full .....	.66	.70	ns
	Decreased .....	.35	.36	
% N in juice.....	Full .....	.064	.066	.012
	Decreased .....	.122	.140	
% K <sub>2</sub> O in juice.....	Full .....	.145	.222	.017
	Decreased .....	.187	.312	
Total dry wgt. (grams)....	Full .....	1121	1130	ns
	Decreased .....	801	795	

(ns = no significant interaction.)

The only significant interactions between sunlight and potash are those concerned with the concentrations of nitrogen and of potash in the crusher juices. With full sunlight conditions, the extra potash apparently had no influence on the per cent N of the juice, but under decreased light the extra potash application has definitely increased the nitrogen content. These data also show that the per cent K<sub>2</sub>O in the juice was influenced to a greater degree when extra potash was given to plants grown under decreased sunlight than when full sunlight conditions prevailed.

There is apparently no evidence in these data that under the poorer sunlight conditions an extra application of potash could be made to partially compensate for the deficiency in sunlight.

*Interaction between Potash and Nitrogen:*

TABLE VI  
(Averages of 8 Pots)

Measurement	Potash	Average N	High N	Excessive N	Difference required
Lbs. cane .....	Average .....	4.29	4.39	4.47	.40
	Extra .....	4.41	4.78	4.27	
Purity .....	Average .....	89.4	83.7	78.8	2.2
	Extra .....	88.6	85.6	80.6	
Y % C.....	Average .....	13.53	10.94	9.29	.76
	Extra .....	13.02	11.73	9.94	
Lbs. sugar .....	Average .....	.58	.50	.44	.06
	Extra .....	.58	.58	.44	
% N in juice.....	Average .....	.030	.068	.180	ns
	Extra .....	.034	.076	.199	
% K <sub>2</sub> O in juice.....	Average .....	.18	.17	.15	ns
	Extra .....	.28	.27	.26	
Total dry wgt. (grams)....	Average .....	956	968	958	ns
	Extra .....	947	995	945	

(ns = no significant interaction.)

Although not a part of the primary objective in this study, the influence of potash in reducing the deleterious effects of high nitrogen applications is nevertheless interesting. This is chiefly concerned with its effects upon the juice quality. The purity of the crusher juice and the yield per cent cane were not as adversely affected by the high nitrogen application when extra potash was supplied; hence the loss of sugar for high nitrogen over average nitrogen which occurred when only average potash applications were made was not apparent when this high nitrogen was accompanied by extra potash.

#### *Conclusions:*

In this study of the effects secured from applications of nitrogen and potash fertilizer in amounts above what were considered adequate for minimum requirements of sugar cane, we have measured the influence of a sunlight deficiency upon these effects.

The interactions between sunlight and nitrogen are much more definite than those few significant interactions which were found between sunlight and potash.

It appears quite clear that in an environment where sunlight is apt to be limited, decreased sugar yields will result if nitrogen applications are too high. Juice analyses indicate that this extra nitrogen is taken up by the plant but is apparently not assimilated.

No evidence was secured to indicate that extra potash applications would prove beneficial where inadequate sunlight conditions exist.

If extra nitrogen applications are to be given, then it would appear wise to accompany them with extra potash since there appears to be a point of balance between these two nutrients which can have its effect especially upon juice quality.



## A Devastating Weed

By E. L. CAUM

There has very recently been introduced into Fiji and Samoa a vine which, brought to Hawaii either by accident or design, would become a pest of major importance. It is one of the rather numerous company known as "mile-a-minute" vines, and one to which, from all accounts, the name is exceptionally well suited. Two native Samoan names have been recorded, *fue saina* and *fue sega* (*senga*), neither of which seems to have any particular significance as applied to the plant in question. It is not improbable that both are erroneous transcriptions for *fue sina*, the name of a large rampant bindweed, *Ipomoea alba*. In gross appearance, if the flowers are disregarded, the two vines are somewhat similar, and the transference of the name of a well-known native plant to an immigrant species resembling it in a general way is frequently done in Polynesia.

Known botanically as *Mikania micrantha* HBK, the plant is native to Central America but, now almost pantropic in range, it is undoubtedly the commonest of the almost three hundred species of shrubs and vines which make up the genus *Mikania*. They are all of American origin, and with the exception of this one species, they seem to be confined to the Americas. This species, usually identified as *Mikania scandens* but which more probably is *M. micrantha*, is widely distributed throughout tropical and subtropical America, and has been reported from Burma, Siam, the Malay peninsula, the Philippines (but not in the neighborhood of Manila), Java and most of tropical and subtropical Africa, as well as Fiji and Samoa. In the West Indies the plant is called "climbing hempweed," a name applied in the eastern United States to a closely allied species, the true *M. scandens*.



Fig. 1. *Mikania* completely blanketing the ground and low-growing vegetation near Matavanu crater, Savai'i, at an elevation of about 1,600 feet. (Photograph by E. H. Bryan, Jr., courtesy Bernice P. Bishop Museum.)

*Mikania micrantha* is a large rank-growing herbaceous vine, abundant in thickets, along roadsides and in abandoned or uncared-for clearings, as well as in more open places in the forests. The stems are slender, generally smooth but sometimes slightly hairy. The leaves are opposite or paired at the nodes, thin, wavy-edged, pointed at the tip, the base sometimes rounded or somewhat wedge-shaped but generally two-lobed with a deep, broad sinus between, 2 to 4 inches long by 1 to 2 inches broad, conspicuously three-nerved from the base, and smooth or practically so. The flower heads, borne in clusters on the ends of the branches or in the leaf axils, are white or slightly brownish, and greatly resemble those of the well-known *pamakani*; the clusters are usually from 2 to 4 inches in diameter, and are said to have an odor of vanilla. The seeds, like those of *pamakani* and *pualele*, have tufts of hairs at the tip to aid in dispersal. The drawing, made from Samoan specimens in the collection of the Bishop Museum, clearly shows the appearance of the plant, and the enlarged inserts picture a single flower head and a single immature seed with its parachute and the floral parts. These latter break off when the seed is fully mature.

Mikania forms dense tangles completely covering the ground and climbing over and smothering out the ordinary roadside shrubbery and small trees. In Samoa it is known to kill even large breadfruit trees, and abandoned or untended coconut and banana plantations are rapidly choked out. Should it come to Hawaii, its potentialities for harm are obvious and enormous. It is a near relative of *pamakani* (*Eupatorium glandulosum*), but because of its climbing habit and its more vigorous and rampant growth, it would easily outstrip that open-land pest as a cause of damage and loss to the ranchers of the Territory. The tiny seeds, slightly more than 1/20 of an inch long, are formed in vast numbers, and each is equipped with a little parachute of fine white or tawny hairs. Borne by the wind, from a base in an open uncultivated field, in wasteland covered with lantana scrub, or in a guava thicket along the lower edge of the forest, these parachute troops could easily invade the forests much more effectively than the Hilo grass and Uluhi fern have done, and with results far more disastrous.

Not only the ranches and the forests, the banana and papaia plantations, but the cane fields of the Territory would suffer severely by the establishment of this vine in Hawaii, forming as it does a dense, solid mat over everything it encounters. With our two-year growing period, vines allowed to grow in our cane fields could easily account for immense losses, not only in actual cane smothered out and destroyed, but in increased cost of harvesting what was left. Neglected for a short time and allowed to form seed, the vine would present a problem well-nigh insuperable. The potentialities for evil inherent in this plant are so much greater than in any weed with which we now must contend that comparisons are meaningless, but it may be stated with perfect safety that, were the vine growing in the gulches and wastelands adjacent to the cane fields, the everlasting vigilance necessary to prevent its spread to the fields would be an item of absolutely major importance.

We now have growing in the Islands vines of several species that are almost as luxuriant in growth as this *Mikania*, but none of them propagate and spread with anything approaching the ease and facility that it does, and hence they have not, and in all probability never will become of any particular significance economically. It is in just this ease and facility of spread that the danger lies. From accounts

by persons who have seen it in Samoa, it is a plant that is rapidly taking complete possession of the country.



Fig. 2. *Mikania micrantha* HBK.

While we have no direct evidence of the date of its introduction into Samoa, we have some indirect evidence that is significant. Dr. F. Reinecke, who made botanical collections in Samoa between 1893 and 1895, does not record the plant in his "Die Flora der Samoa-Inseln," and Dr. W. A. Setchell, who collected in American Samoa in 1920, likewise makes no mention of it in "The Vegetation of Tutuila Island." When experienced field botanists like these two did not find the plant, we are justified in assuming that in all probability it had not reached Samoa in Reinecke's time, and if present at all on Tutuila in 1920 it was exceedingly rare.

The first published record of the occurrence of *Mikania* in Samoa was made

by Dr. Erling Christophersen in "Flowering Plants of Samoa," published in 1935, on the basis of collections made by himself and others between 1921 and 1932. The first collection cited was made by E. H. Bryan, Jr., in 1924 on Upolu, Western Samoa. Mr. Bryan visited the larger islands of the Samoan group in that year, and in conversation has stated that in his opinion the vine, if it was present at all on Tutuila, was not sufficiently common to be noticed, and it is far from inconspicuous. On Upolu it was locally common in the neighborhood of Apia, but had not reached the rain forest. On Savaii it was the dominant factor in the lower forests on the north side, behind Safune and Matautu Bays, being everywhere exceedingly abundant, growing over and smothering out all the vegetation it encountered. Apparently it had not at that time crossed the high peaks to the south side of the island, as no trace of it was seen there. The general opinion among residents was that it had first appeared four or five years previously, or about 1919 or 1920. On his next visits to Samoa, in 1935 and 1938, Mr. Bryan learned that the vine was widespread on Savaii and Upolu, and found it rapidly becoming a major pest on Tutuila. In 1927 Dr. P. H. Buck noted that on the south side of Savaii, where there had been little or no Mikania in 1924, several coconut plantations had been abandoned because of it. No method of control had been found that could even keep pace with the rapidity of growth and spread of the vine. The foci of infection on these three islands were apparently the ports of entry.

We do not have the dated information on the status of the pest in Fiji that we have concerning it in Samoa. Mr. Bryan saw it in 1924 completely covering cliffs and bluffs along Suva Bay, and E. C. Zimmerman, who traveled in Fiji in 1938, has said that a warning against the introduction of this plant into Hawaii cannot be made too strong.

Our knowledge of the serious effects resulting from the establishment of Mikania in Samoa is more detailed than it is concerning the other regions to which the vine has migrated, and for that reason and because of the physical similarity with Hawaii, Samoa has been emphasized. However, it is not only in the Pacific Islands that the plant is making its presence felt. To translate very freely from Backer's *Onkruidflora der Javasche Suikerreitgronden* (Weeds of the Javanese Cane Fields)—In flower January to December. East and West Java, from the plains to an elevation of about 5000 feet, principally in regions exposed to easterly winds, growing in the sun or in part shade but not in dry areas, in scrubby thickets, on ravine walls and the borders of the forests, in young second-growth woodlands and on river banks, local but widely scattered, sometimes very frequent, often forming great confused tangles.

All the information available shows the plant to be capable of extremely rapid spread, particularly when aided by the absence of natural enemies, and despite attempts at mechanical control. Our necessarily stringent preventive quarantine against immigrant insects is efficient and effective, but the possibility of the immigration of plant pests must not be overlooked. This one, very abundant in two island groups only a few days from us by steamer, might easily reach here as stray seeds caught in baggage or on clothing—the many lauhala mats imported from Samoa would be excellent for transporting the seed—and it behoves us, as a matter of self-defense, to watch carefully for it and to eliminate promptly any plants that

might possibly get started. A chance seed, germinating and growing, ignored as just another weed, might well be enough to afflict us with a plant pest beside which those we now have would be negligible.



Fig. 3. A view immediately behind the Naval Station at Pago Pago, Tutuila. (Photograph from O. H. Swezey.)



Fig. 4. A view from behind the Naval Station, looking across Pago Pago Bay toward the Rainmaker mountain. In this figure and in No. 3, the light-colored areas on the mountain sides are masses of the Mikania vine. (Photograph from O. H. Swezey.)



Fig. 5. *Mikania* completely swamping the roadside vegetation and climbing into the trees, near Malololelei, Upolu, at an elevation of about 2,000 feet. (Photographs by E. C. Zimmerman.)



Fig. 6. *Mikania* in second-growth scrub on abandoned land near Nasori, Fiji. (Photographs by C. E. Pemberton.)



## Colchicine in Relation to Sugar Cane Breeding\*

By D. M. WELLER

So much has been published about the effects of colchicine† on plants that a certain degree of popular interest has been developed in it. Although the subject has been treated extensively in articles and reviews both from the practical as well as from the theoretical aspect, there have arisen in the mind of the layman certain misconceptions about the purpose of its use, its action, or the results of its action.



Fig. 1. Side and top views of two flower heads of marigold. The larger is from a tetraploid branch of a plant which had been treated with colchicine when a young seedling. The smaller one is from a normal, untreated plant.‡

\* Presented before the Agricultural Section of the Hawaiian Sugar Technologists, December 12, 1939.

† Colchicine is an alkaloid drug. It is sold as a yellowish powder which is readily soluble in water. It is highly poisonous.

‡ Reproduced by permission. Nebel, B. R., and Ruttle, M. L., 1938. The cytological and genetical significance of colchicine. *The Journal of Heredity*, 29: 3-9 Frontispiece.

It has sometimes been thought that the use of colchicine will develop into a chemical short cut for doing what the plant breeder is now doing and that eventually it will completely supplant his work. This is entirely erroneous; however, for it can become for the geneticist no more than one of his tools. It is true that with it, as with any specialized tool, he may accomplish something that he might not otherwise be able to accomplish, but the materials to be used, *i.e.*, the plants to be treated, may be the product of his own work, and the results obtained and their further development and application are still his. Any apparently desirable, or "changed," plant resulting from colchicine treatments must be tested by the same methods and by the same standards as are varieties produced by the classical method of plant breeding. In order to know whether the plant being dealt with is a diploid, triploid, or tetraploid, etc., its chromosome number must be determined and also, both its commercial value and its potentialities as a parent must be determined.

Let us look first at some of the results of colchicine treatments obtained with other plants. In Fig. 1 are shown two marigold flower heads. The one on the left was produced on one of the branches of a plant, which was treated with colchicine when it was a small seedling. This treatment was a very simple one consisting merely of inverting the seedling (and the pot in which it was growing) and immersing its top for several hours in a 0.16 per cent aqueous solution of colchicine. The flower head shown on the right grew on a normal untreated plant. The larger size of petals and of flower head as well as the greater general plant vigor as a result of the colchicine treatment are apparent in the one on the left.

Colchicine treatments are effective, therefore, in changing a plant into a plant of a different kind, the differences between the two being similar to those differences which exist between parents and their hybrids when polyploidy has been produced but which have been brought about by a chemical method.

Such spectacular and practical results obtained so easily are only to be wondered at.

Similar results have been obtained with many kinds of plants. Nebel and Ruttle (3, 4) at the New York State Agricultural Experiment Station, Geneva, N. Y., are succeeding in changing almost every kind of plant with which they work. Blakeslee (2) of the Carnegie Institution of Washington has published a list of names of about 100 kinds of plants which have been altered by this method. Meyers of the United States Department of Agriculture and Sears (5) of the United States Regional Laboratory for Pasture Research at State College, Pennsylvania, have changed pasture grasses, wheat, etc., of the *Gramineae* to which family sugar cane belongs. There seems thus to be no reason why sugar cane should not also be capable of improvement by this chemical method.

The explanation of the differences between the two marigold plants, which produced the two flower heads pictured in Fig. 1 (and also the changes in the other plants mentioned above), is to be found in the fact that the number of chromosomes in the cells of the branch which produced the larger flower head had been doubled by the colchicine treatment and that it had become what is known as a *tetraploid* plant (in this case, however, it was only one branch rather than the whole plant that was affected by the treatment), while the number of chromosomes in the cells of the plant which produced the smaller flower head had not been doubled but had remained of the same number as that of a normal *diploid* plant.

The results of colchicine treatments are, therefore, changes of morphological, physiological and genetical characters which accompany the fundamental change from a diploid plant to a polyploid plant (usually a tetraploid). As compared with diploid plants, tetraploid plants are usually larger in size, are more robust and possess greater vegetative vigor. They produce larger flowers and fruit and are characterized by possessing larger stomata and larger pollen grains than diploids. Much of our present-day agriculture is based upon the use of "improved varieties" by which is often meant hybrids which are tetraploids.

Wild tetraploid hybrids occur in nature but their occurrence is comparatively rare, the frequency of their occurrence depending upon the nature of the plant itself and the conditions of its ecological association. They occur more frequently in the populations of the plant breeder where the method of the selectionist, such as Luther Burbank, while laborious, expensive, and haphazard, nevertheless, produced spectacular results. The laws of Mendel made it possible to work along preconceived lines in an orderly manner which was less expensive. According to some geneticists (1), the limitations of Mendelian methods have been reached for many of our common crop plants because the number of possible combinations of characters have been exhausted and a point has been reached where the hybridizer can only "wait for some new mutation to turn up." (Certainly, however, this is not true for cane.) Therefore, newer and more direct methods have been sought.



Fig. 2. The  $F_1$  wheat hybrid (second from left) is sterile and produces no seeds. If the seeds, which develop into such sterile hybrids, are soaked in a colchicine solution before being planted they develop into new types of plants which produce fertile flowers and set seeds (third from left, with the seeds it produced shown above it). The two parent wheats are shown on the extreme right and left.\*

The new type of plant owes its fertility to the fact that it is a *tetraploid*, the colchicine treatment having changed its chromosome number from the  $2n$  to the  $4n$  number.

\* Reproduced by permission, Sears, E. R., 1939. Amphidiploids in the *Triticinae*, Induced by Colchicine. *The Journal of Heredity*, 30: 38-43.

Such methods attempt a controlled artificial production of new plant types, especially those which are polyploids. Among these methods have been the use of X-ray radiations, centrifuging, decapitation of apical shoots of seedlings, thermal treatments, etc., but their success has been meager. It is not surprising then that the hope, which the use of colchicine holds of fulfilling this need, has resulted in a wave of popularity of the use of this chemical method as a promising "non-Mendelian" method because it has with many varieties of plants so greatly increased the rate of occurrence of tetraploid plants as to extremely reduce, if not entirely eliminate, the factor of chance.

In addition to inducing *gigas* characters in plants, another practical use to which colchicine is being put is that of changing sterile hybrids into fertile ones. Plant breeders of the United States Department of Agriculture have produced fertile  $F_1$  wheat hybrids from crosses which formerly had always resulted in sterile ones.

In Fig 2 is shown a sterile  $F_1$  hybrid (second from left) of two wheats (*Triticum monococcum*  $\times$  *Aegilops uniaristata*). The two parents are shown on the extreme right and left. If the seeds which ordinarily develop into such sterile hybrids are soaked for 24 hours in an aqueous solution of colchicine they develop into

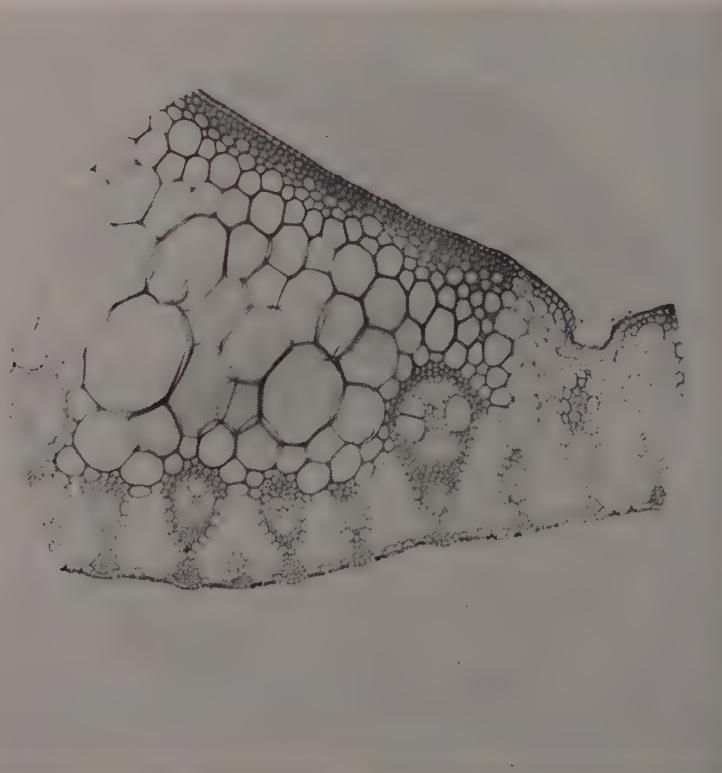


Fig. 3. A photomicrograph of a thin slice of a sugar cane leaf at the junction of the midrib and blade showing that a plant is composed of cells arranged in groups or layers called tissues.  $\times 75$

plants which are fertile and set seeds (third from left). In these plants the chromosome number was found to have been doubled so that they are now tetraploids. Differing from the tetraploid marigold plants, however, they were not changed from their parents in vegetative characters.

These two examples illustrate, therefore, that tetraploidy induced in plants by colchicine treatments may be expressed by increase of size and vegetative vigor, or by fertility of flowers and the setting of seed unaccompanied by an increase of vegetative vigor.

To understand the use of colchicine, it is first necessary to understand something about *cell division, chromosomes, and inheritance*.

When a thin slice (section) of a sugar cane root, or leaf, or any part of the plant is examined under the microscope, its texture is seen to be lacy or porous. See Fig. 3.

This is due to the fact that, as everyone knows, plants (and in fact almost all organisms) are made up of *cells* and that the circular outlines seen in the section are the outlines of the slices of these more or less globular cells and their contents. It will be seen further that these cells are arranged more or less in layers, each layer being made up of the same kind of cells. Such layers are known as *tissues*. A tissue is composed of cells of similar origin, of similar structure and of similar function. There are many kinds of tissues with the cells of each differentiated in structure in adaptation to specialized function. Cells of certain tissues have thin walls and are colorless while those of others are green because they contain chlorophyll; cells of still other tissues have no cellular contents but their walls are thickened and hardened and they are joined together end to end with their end walls dissolved out in such a way as to form tubes for water conduction.

The growth of a plant is due to the enlarging and expanding of its cells very early in its development and to the division of its cells into others which do likewise.

It is with this latter process—the *division of cells*—with which we are concerned in dealing with colchicine.

There are two types of cell division: *somatic*, or vegetative, cell division, called *mitosis*, and *meiosis*. The first of these, mitosis, is the one involved in the growth of tissues mentioned above. The second, meiosis, has to do with the formation of sex cells such as are contained in pollen grains and fertilize the egg cells contained in the ovules, which then develop into the seeds of the plant. Only the first one of these types of cell division will be considered briefly.

When the cells of very young plant tissues which have been properly stained are examined under a higher magnification than those shown in Fig. 3, they will be seen to have at or near their centers a prominent spherical structure known as the *nucleus*. See Fig. 4.

Even within these small spherical structures (under proper magnification) are to be seen specialized organs, the most conspicuous of which are the *chromosomes*. According to the stage of development of the nucleus or the individuality of the plant these are long thin threads, short bent rods, or isodiametrical blobs of chromatic material high in protein but whose biochemistry is, as yet, not well known. See Fig. 5.

Their function is well established, however, as the carriers of the hereditary

units called *genes*. While genes are not visible even under the highest magnification, certain markings on specially stained chromosomes are interpreted as being their *loci*. In Fig. 6 are shown chromosome "B" from 13 individual grasshoppers on which knobs or swellings can be seen. Because they are constant on individual chromosomes, each chromosome has a distinguishing morphology by which it can be recognized and identified from individual to individual and from generation to

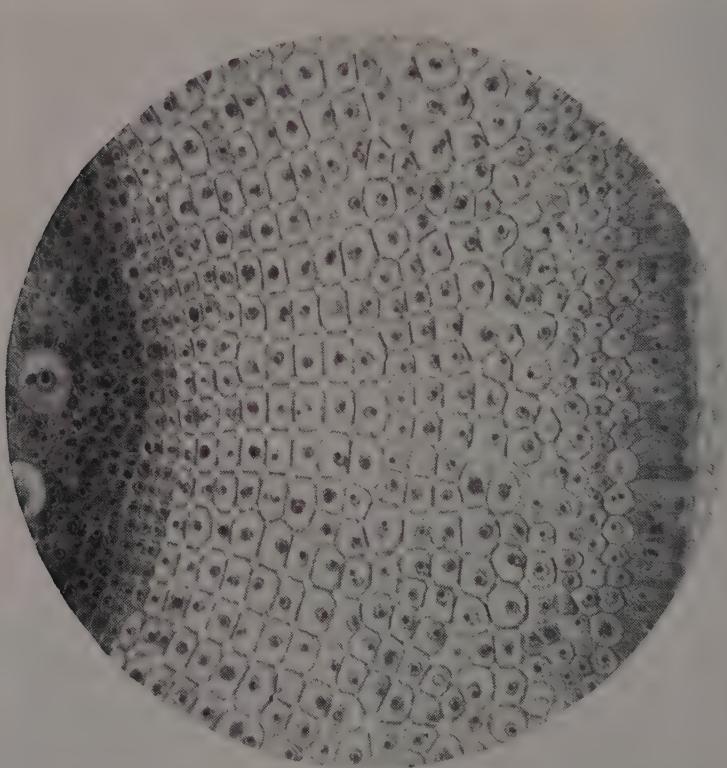


Fig. 4. A photomicrograph of a portion of a transverse section of a root of sorghum (with which sugar cane is sometimes crossed) showing that within each individual cell composing its tissues is a spherical body called a *nucleus*.  $\times 260$

generation. Furthermore, regardless of how many chromosomes a plant may have, they occur in duplicate in the nuclei of vegetative cells but singly in sex cells. Thus we speak of "pairs of chromosomes" as occurring in somatic cells and of such cells as having two sets, or *complements*, or as having the  $2n$  number of chromosomes (*diploid*). In the sex cells there is but one set, or complement, or the  $n$  number (*monoploid*). A *tetraploid* plant, therefore, is one with four sets of chromosomes in its vegetative cells and two sets in its sex cells instead of *two* and *one* as is usual; and there may be *triploid*, *tetraploid*, *pentaploid*, etc., and higher

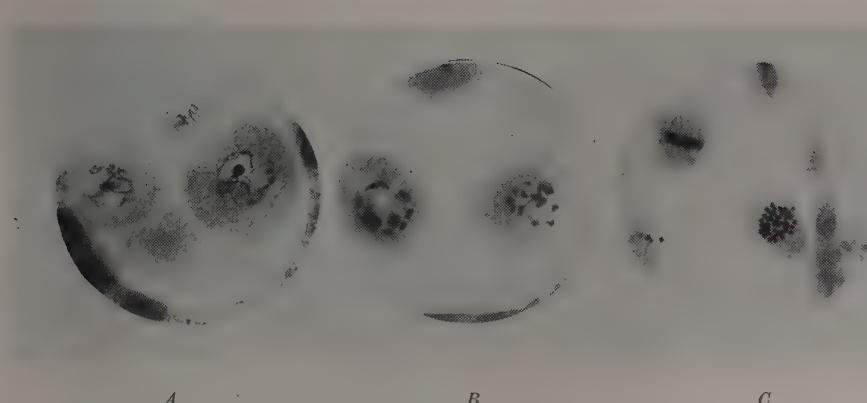


Fig. 5. Showing how the chromosomes within the nucleus of a cell at different stages of its development may assume different forms. (*Microsporocyte* of sugar cane.)

*A*, showing them at an early stage of development in the form of long thin threads on which in many plants knobs of chromatin may be seen (*prophase*).  $\times 588$

*B*, and *C*, showing them at later stages in the form of contracted, more or less isodiametric blobs, or spherical bodies, of chromatic material (*late prophase* and *metaphase*).  $\times 884$



Fig. 6. Chromosome *B* from 13 individual grasshoppers (*Phrynotettix magnus*) showing constancy in size and arrangement of the principal "knobs" (chromomeres), the loci of some of the genes or "inheritance units." The same constancy exists in the different cells of a single individual.

Chromosomes may be found again and recognized in the several individuals of the offspring of an organism and from generation to generation they maintain their identity. (Redrawn after Sharp.)

multiples designated as *11-ploid*, *12-ploid*, etc. These terms apply to the cells, tissues, individuals, races, or species with such nuclei.

When a vegetative cell divides, the chromosomes of its nucleus split longitudinally and, in the early prophase stage, when they assume the form of long thin threads, may be seen actually as threads of a double nature. Later these two halves become individual chromosomes and assume the form of short bent rods or isodiametrical blobs and then "line up" in pairs opposite each other on an "equator" from which are seen "spindle fibers" extending upward and downward and coming to a focus at a north and a south "pole." Through some influence or force of the fibers or existing within the chromosomes themselves, the members of the pairs separate and move toward their respective poles. After the chromosomes have left their position at the equator, other fibers can still be seen extending across the equator and at the equator a cell wall forms, thus dividing the original cell into two cells. At the same time the two groups of chromosomes organize a new nucleus at each of the poles and become the nuclei of the two new daughter cells. See Fig. 7.



Fig. 7. *A*, side view of a dividing nucleus of a sugar cane cell showing how the chromosomes are lined up in pairs at the equator, each member of a pair being opposite its mate across the line. A "spindle" of fibers crosses the equator converging upward and downward and coming to a focus at a north and south "pole." This stage is known as *metaphase*.  $\times 744$

*B*, a later stage of the same showing that the members of the pairs of chromosomes have separated from each other and moved to opposite poles where they are organizing two new nuclei. At the equator spindle fibers are still visible where is taking place the formation of a cell wall which will divide the original cell into two daughter cells. This stage is known as *telophase*.  $\times 558$

It is with these spindle fibers that colchicine has to do, for by some as yet unexplained chemistry it prevents not only their formation, but the separation of the paired chromosomes, and the formation of the dividing cell wall as well. The result is that the two separating groups of chromosomes, now double their original number, remain in the original cell already enlarged preparatory to becoming two cells, and organize a new nucleus greatly increased in size because of the doubled number of chromosomes. In subsequent divisions such cells are consistent in dividing into daughter cells having the doubled number of chromosomes.

It is readily seen, then, that if the meristematic cells of the growing point of a

stalk or of a bud or of a seedling have been affected by colchicine, a stalk or a branch will develop from it in which the nuclei of the cells may contain double the number of chromosomes, or four *complements*. Such stalks or branches, therefore, are *tetraploid* which explains the increase in size of the marigold shown in Fig. 1 and the other changes mentioned above. It is possible, of course, for the colchicine treatment to affect the meristematic cells in such a way that other multiples of the basic chromosome number result. It is possible to produce plants which are monoploids, triploids, tetraploids, etc., and higher multiples such as 11-ploid, 12-ploid, etc. Plants of many genera have already been reported to have been affected in one or another of these ways.

When the second type of cell division, namely, *meiosis*, is considered, it is easily seen that, in the case of a tetraploid, for example, pollen grains and egg cells may have twice the number of chromosomes they ordinarily have, i.e., they contain two sets of chromosomes instead of one as is normal. A *diploid* egg fertilized by a *diploid* sperm would result in a *tetraploid* hybrid.

Applications of colchicine to sugar cane have yielded interesting responses.

Several thousand sugar cane seedlings developing from colchicine-treated seeds of some fifty hybrids within the genus *Saccharum* showed various effects. Below the lethal treatment, which differed for different varieties, stimulation or depression of growth resulted according to concentration of solution and duration of treatment. Shoots developing after treatments with high concentrations were short, broad, globose, or sometimes differed little in external appearance from the normal (Fig. 8).



Fig. 8. Germinating sugar cane seeds.  $\times 10$   
A, showing a normal *coleoptile* (shoot) developing from an untreated sugar cane seed (control).

B, showing a broad, globose *coleoptile* developing from a sugar cane seed which was soaked for three hours in a 0.16 per cent colchicine solution.

Cytological studies of such seedlings showed the cells of their coleoptiles and growing points to contain lobed nuclei or multi-nuclei (Fig. 9). Division figures exhibited abnormal spindles and an increased number of chromosomes staining more deeply than normally.

Seed treated with aqueous solutions of colchicine showed not only an increase

in percentage of germination but also a stimulation of growth of seedlings (Fig. 10).

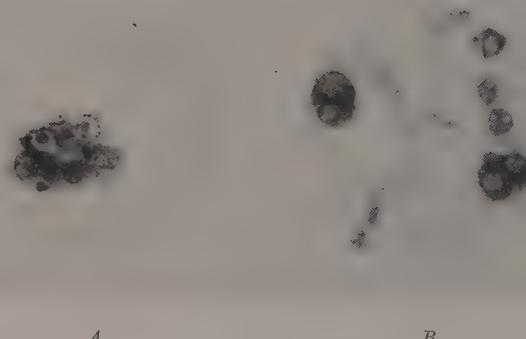


Fig. 9. Cells of growing points of sugar cane seedlings which after treatments with colchicine contain lobed nuclei (*A*) and two or three nuclei (*B*) instead of a single, smoothly globular nucleus as is normal. *A*,  $\times 774$ ; *B*,  $\times 558$



Fig. 10. Showing stimulation of percentage of germination of sugar cane seeds as well as stimulation of growth of seedlings as a result of colchicine treatments. (Flats numbered from left to right.)

Flat	3-hr. treatment	% germination
No. 1.....	Distilled water (Control)	19.0
" 2.....	0.04% colchicine	25.0
" 3.....	0.08% "	36.0
" 4.....	0.16% "	21.0

Very young buds near the growing points of stalks of mature cane developed into shoots to all appearances differing little, if any, vegetatively from the parent plant. However, flowers of such shoots produced pollen grains several times as large as normal pollen grains (Fig. 11).

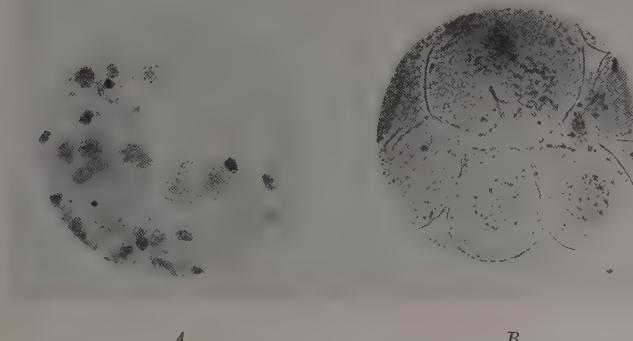


Fig. 11. Showing pollen grains of sugar cane of the variety Mol. 1032.  $\times 558$   
*A*, normal pollen grains from flowers of a tassel of a control plant.  
*B*, pollen grains from flowers of a tassel borne on a stalk developing from a bud which was treated with colchicine several months previously. Note the great increase in size and the two groups of chromosomes in the upper pollen grain.

Such "referred" effects may be quite as practicable and valuable to the plant breeder as direct effects. Often effects of colchicine, either a stimulation or depression of growth, were produced in stalks developing from eyes (on the same seed piece) other than those treated. This was especially true if the dosage was lethal or nearly lethal and a depression of growth of the treated eyes resulted. In such cases the shoots developing from eyes either above or below the treated one expressed the effects showing that the effects of colchicine treatments can be referred either upward or downward in the stalk.

These changes are proof that colchicine is capable of changing the cellular and nuclear organization of the cane plant as it has been found to be for other agricultural plants and its use with the cane plant needs only to be understood and controlled to be helpfully effective.

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# The Factor of Synergism in Chemical Weed Control

(A consideration of some of the other problems of weed eradication.)

By FRANCIS E. HANCE

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## INTRODUCTION

A review (2) of the literature of chemical weed control was presented in 1938. At that time, and now, there does not exist any entirely satisfactory method of controlling weed growth on agricultural lands by the application of liquid chemical spraying compounds.

Nevertheless, progress has been made recently in research studies on the topic by Hawaiian plantation staffs and by members of this Experiment Station. It is the purpose of the present article to present a discussion on improved practices and formulas now used in Hawaiian chemical weed control as a result of the studies made during the past two years.

#### COMMONLY EMPLOYED SPRAYING MATERIALS

##### *The Item of Cost:*

It is recognized that cost consideration is a first requisite in selecting any herbicide. Delivery charges, containers, proximity to source of supply, hazards in transit and concentration of toxic principle must be considered in computing costs. As an illustration of this point we might cite the ease of procurement and low purchase price of tank car shipments of factory sludge acids to nearby agricultural regions on the mainland. In Hawaii there are no suitable industrial wastes to be obtained, at any cost, which have herbicidal value.

##### *Four Classes of Herbicides:*

Actually there are but four classes of chemical herbicides which are economically available for large-scale application: (1) Compounds of arsenic, (2) the chlorates, (3) petroleum products, and (4) sulfuric acid. Any of these products may be "extended" or diluted with water. It is this property of miscibility with water that makes them economically available to Hawaiian users.

##### *Objections to "Economically Available" Herbicides:*

Considering them in the order listed, the disadvantages of the four classes of weed killers are many and, in some cases, serious.

*Arsenic:* Arsenic is poisonous to men and animals. The dangers involved in handling the chemical, in preparing it for spraying and in avoiding accidental injury from its solution are always operative and as a consequence, in respect to its poisonous properties, arsenic cannot be classed as a satisfactory herbicide.

Another property of arsenic, when dissolved in a solution of alkali and water, or acid and water, or water only, concerns the fact that it becomes fixed in the soil to remain there and to be added to almost arithmetically in successive sprayings. Eventually, of course, if the practice is continued, the accumulation of fixed arsenic must impair the agricultural value of the soil.

*Chlorates:* In the literature review referred to previously (2) the behavior of chlorates in weed control is discussed in detail. Summarizing this discussion, and adding our recent experiences, it is apparent that the chief objections to chlorates include soil sterilization, fire hazard, a cost of 8 cents per pound for sodium chlorate and a requirement of about 10 per cent concentration of chemical in solution for satisfactory phytocidal effect.

*Petroleum Products:* Several proprietary petroleum herbicides and ordinary Diesel oil give excellent results in destroying the general run of field weeds. The disadvantages of these oils for use in Hawaii are: higher first cost per unit of lethal effectiveness based upon bulk of material as purchased; the fact that these substances do not exist in highly concentrated form; and furthermore, that dilution

of original material with water in the field is seldom advisable, but if dilution may be in order, it requires an emulsification operation and not simple solution.

*Sulfuric Acid:* There are a number of disadvantages to be overcome in using this mineral acid as a herbicide. A commercial grade of 60-degree acid could be manufactured in large quantity and supplied for about \$17.00 per ton at the Honolulu plant of The Pacific Guano & Fertilizer Company. A similar grade of acid from mainland sources would cost about \$30.00 per ton. It would prove a problem to transport this corrosive liquid to field points and it is obviously unsafe to trust its dilution to any other but trained men. It could not be used in ordinary spraying equipment because of its corrosive character. At a dilution of from 5 to 10 per cent of concentrated acid (the ratio used) the solution as a spray would be objectionable if accidentally blown into the face or otherwise brought in contact with external or internal body parts. This acid would not "poison" the soil, but claims have been made by other workers that when applied to the land over a long period of time a tendency exists to deplete soil bases by chemical combination, followed by solution of the salts produced and subsequent loss through drainage.

#### *Critical Comparison of the Four Types of Chemical Herbicides:*

Of this quartette (arsenic, chlorates, oil and acid) arsenic produces the cheapest and most potent herbicide spray, but its disadvantages are the greatest of the group. Next in line of low cost, we find sulfuric acid. However, in addition to its objectionable features, it has not been found as effective on a comparative cost basis as any of the other substances.

The oil preparations are third on the list of increasing costs. This factor alone has discouraged extensive experimental trial of the oils, but eventual utilization of these materials in effective combination with other agents is a matter we plan to explore in the very near future.

Chlorates, as conventionally used, are the most expensive of this group. The soil sterilization properties of chlorates and their fire hazards are serious hindrances to their use. However, the advantages to be realized in adopting a chlorate program of chemical weed control are so many and so outstanding—as will be shown later—that an effort has been made to reduce chlorate costs by chemical booster treatment and at the same time eliminate induced soil sterilization and the hazards of fire by the further dilution of chlorates made possible in the booster scheme.

#### *The Chlorate Situation:*

There may be prepared today very few herbicide solutions which are superior in general weed destructive properties to one in which 100 pounds of sodium chlorate are dissolved in sufficient water to make a total volume of 100 gallons. As a spray this solution is deadly in its effect on most all plant foliage. As a drench applied to the soil around individual bushes, trees or patches of smaller plants it is equally effective.

To reduce the danger of fire it is common practice to add 100 pounds of calcium chloride to the formula cited above. Unfortunately, this chlorate-chloride solution costs about \$14.00 per 100 gallons. It could not possibly be used freely on weeds within a growing field of cane (or any other crop) because of its sterilization effect on the soil and also because of its stunting and chlorotic effect on the current

crop. This physiological influence is exerted apparently through crop root systems by reabsorption from treated soil. It is significant to note the fact that even though care be observed so as not to apply the spray solution directly upon the crop the latent injury resulting from the practice is perhaps more severe in an indirect way than a deliberate or direct spraying of the crop foliage.

*The Modification of a Chlorate Herbicide Intended to Minimize its Objectionable Features:* In field studies conducted by Q. H. Yuen and the writer in abandoned cane areas rank with weeds, it was found that concentrations of sodium chlorate in water at or under 20 pounds of the chemical per 100 gallons and sprayed on solid weed growth in and about the cane at the rate of 500 gallons per acre it did not occasion the appearance of chlorosis in the cane leaves and did not sterilize the soil as far as could be determined during several months of observation.

It was found in this study that weeds which had been destroyed or severely injured by the chlorate treatment were, within a few weeks, replaced by the appearance of new growth from seed or by recovery of persistent species from the setback suffered.

For example: A single sprig of honohono or portion of a branch stem of pig weed which may have escaped the applied spray would constitute a nucleus for new growth. In a very short period of time such nuclei would develop a vigorous and luxuriant stand almost as extensive as the original infestation.

In other areas supporting succulent annuals which had been entirely destroyed by the treatment, a new growth from dormant seed appeared which reestablished the region with a blanket of verdant weedy pests.

The purpose of emphasis, in this case, is to show that absolutely no evidences of soil sterilization had appeared as a result of applying this low concentration of chlorate to weeds and soil.

On the other hand, from 50 to 100 pounds of sodium chlorate dissolved in 100 gallons of water and applied at the rate previously stated did definitely affect the cane crop and did induce more or less soil sterilization in these higher concentrations.

The damage to the cane from the heavier applications of chlorate was definite and specific. The matter of soil sterilization, on the other hand, was clearly indefinite or erratic. These observations were considered important because they appeared to confirm published experiences of other workers in which a vigorous drenching of the soil was considered essential to induce sterilization in one treatment. Additional importance was attached to the observations in that the sensitivity of the cane plant to graded chlorate treatments of weeds in or about it was defined rather sharply by degree of concentration of chlorate used—all other factors being equal. Cane does not appear to be tolerant of chlorates at concentrations higher than 20 pounds per 100 gallons.

*Dilute Chlorate Solutions Sprayed on Weed Foliage are Ineffective Herbicides:* It soon became apparent in this study that by reducing the chlorate concentration in the weed spraying solution to a point which obviated its flagrantly objectionable effect on crop and soil resulted in a rather indifferent herbicidal effect on several weed types, especially the more persistent ones. But, in spite of this fact, it

was also apparent that even a modest intensification of lethal weed action in these dilute solutions would most certainly improve their herbicidal value.

#### THE SYNERGETIC FACTOR

The word "synergism" implies working together as opposed to "antagonism." The term is used quite commonly in theology and medicine. To illustrate the use of the term in this article, it may be stated that full lethal doses of each of the alkaloids such as morphine and digitalis, when administered together, may fail to cause death. In such a case chemical antagonism has occurred and as a consequence one poison has neutralized or destroyed the action of the other. Conversely, if about one tenth the lethal dose of white arsenic and about one fifth the lethal dose of bichloride of mercury were administered simultaneously the fatal consequences would probably not only be more deadly but more rapid in action. Thus, in the latter illustration the arsenic and the bichloride have acted "synergistically," i.e., have worked together in producing a desired effect faster and more deadly than either compound could do when administered singly and in much stronger doses. One might conclude, therefore, that the arsenic functioned as an "activator" of the bichloride. In this article the terms "activator" and "activation" are used in this sense.

In an earlier research project seeking the development of an organic herbicide, a crude cresylic-phenolic liquor was chlorinated to saturation and then neutralized with sodium hydroxide. Later, a salt was recovered from the resulting reaction residue which was found to consist essentially of a water-soluble chlorinated phenate.

In field tests of this compound as a weed killer we were gratified to find it an excellent herbicide but were disillusioned in finding that 30 or more pounds per 100 gallons of water were required for satisfactory results (about \$7.00 or \$8.00 for the chemical). Additional study revealed the fact that when combined in small quantity with many common weed toxins this chlorinated phenate imparted a property of activation to the spray solution which did not become dissipated even when diluted in water up to 8 times in excess of the regulation killing strength of the weed toxin used. Furthermore, in the presence of an equally small quantity of an inexpensive oxidizing agent this phenomenon of activation or *synergism* became more pronounced.\* Stated more specifically, the addition of about one fourth to one fifth of one per cent by weight of activator and of an oxidizing substance to a herbicide spraying solution enables the user to reduce the concentration of weed killing chemical by from one half to one eighth of that ordinarily used (depending on toxic substance employed and character of weeds) with equally satisfactory or even superior results in lethal effect on the weeds treated.

#### SUNLIGHT AND RAIN INFLUENCE HERBICIDAL EFFICIENCY

Formulas for preparing weeding spray solutions for specific purposes will be submitted later. Before considering the formula matter it may be in order to take

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\* As a matter of protection to this Association an application has been made for a patent on the employment of sodium pentachlorphenate as an activator of chemical herbicides with and without an added oxidizing agent.

note of the very important fact that favorable or adverse weather conditions may enhance or may almost entirely nullify any weakly fortified spraying treatment.

This has been found to be the case, time after time, when using lightly activated and highly diluted spray solutions. In warm, dry weather, under an abundance of sunshine for 3 days following a spraying treatment, a 12½ and 2 formula at 250 (12½ pounds sodium chlorate and 2 pounds activator per 100 gallons applied on solid weeds, knee high, at 250 gallons per acre) would, in Hawaii, reduce a mixed-weed population to brittle, practically dry tinder. The same formula applied in the same quantity in warm, damp weather, followed by intermittent rains for 3 days after spraying, would result only in singeing the weeds. In the latter case recovery of the area and reestablishment of weed growth would be a matter of a week or two. In the former case complete recovery may be delayed for months—provided dry weather continues.

It is necessary, therefore, in large-scale field treatment to use a spraying solution which will function efficiently under conditions midway between the two extremes of weather which were cited.

#### THE "SPREADER" OR SURFACE-TENSION DEPRESSANT

In general, Hawaiian agriculturists are committed to the use of an effective spreader. They have learned by experience that the practice is a paying one. Research has shown that less spray solution is required per unit area of weeds as ordinarily applied when the solution contains the correct concentration of an adequate spreader. Furthermore, waxy, oily leaf surfaces and hairy stems are actually wetted with the killing spray, provided the spreader used will offset the natural tendency of greasy surface or downy stem to agglomerate the applied spray in droplets and discharge it thereafter by gravity to the ground below.

On an acreage field scale basis a plantation on this island (Oahu) recently found it possible to reduce the concentration of weed killing agent by 50 per cent, using the full quantity per 100 gallons of a spreader suited to their conditions as recommended by this Experiment Station. The cost per gallon of the final spray solution plus spreader was a trifle more than the former spray solution without this amendment. However, the reduced concentration of active herbicide, permitted by adding the spreader, and the greater coverage properties of the treated spray compensated, it appears, for the increased unit cost of complete spraying solution.

#### TYPES OF SPREADERS

There are three distinct classifications of commonly used spreaders in Hawaii, i.e., soluble oil, sulphonated ester compounds, and the H.S.P.A. activator (pentachlorphenate), the latter compound serving the dual purpose of spreader and activator.

##### *Soluble Oil:*

As a rule this substance contains an oil-soluble soap, the ingredients of which may be incorporated with a carrier oil, such as the light mineral oils, before saponification is effected. Addition of water and agitation emulsify the prepared oil compound and thereafter contribute to a reduced surface tension of an aqueous

solution to which the soluble oil may have been added. One hundred gallons of water or spraying solution require about one quart of soluble oil for satisfactory performance. The greatest disadvantage of soluble oil is the adverse effect it exercises on, and the resulting much shorter useful life of, rubber gaskets, washers and hose used in spraying equipment. The plantation worker does not always consider this important item when comparing relative costs of various spreaders. The newer, modern soluble oils are excellent spreaders and cost about 55 cents per gallon, or about 14 cents per 100 gallons of final spray solution.

#### *The Sulphonated Ester Compounds:*

In several years of laboratory and field research we have failed to find any spreader the equal of a new chemical described in a U.S.D.A. pamphlet (1) as "a dioctyl ester of sodium sulfo succinate." This chemical is marketed, among other forms, as a paste containing 85 per cent of the original compound and sells for about \$1.00 per pound in 1,000-pound lots. Upon additional dilution to a 5 per cent or a 10 per cent concentration by adding water a usable gelatinous-like solution is obtained which is easily incorporated with the spray solution.

One quart of the 10 per cent solution of the 85 per cent paste imparts highly satisfactory spreading properties to 100 gallons of commonly used spray solution at a cost of about 20 cents per 100 gallons. This cost is about 50 per cent greater than that of soluble oil, but the effect produced is considered superior to the oil and, in addition, it produces absolutely no detrimental effect on rubber parts of spraying apparatus. Another advantage of this chemical ester is its high concentration as received and hence it enjoys cheaper handling and transportation costs to the place of usage. It is non-poisonous (internally or externally applied), bland, odorless, stainless and does not deteriorate in storage.

#### *The Activator as a Wetting Agent:*

Combinations of activator and sodium chlorate in water solution are peculiar in that no added spreading agent appears to be needed. Laboratory study of this property of the combination of these two chemicals is contemplated in the very near future. Incorporated with most other herbicides, the activator does not impart sufficiently reduced surface-tension effects to obviate the necessity of adding other and more active spreading agents.

#### FORMULAS

The so-called standard chlorate formula is submitted below for purposes of comparison. It is to be emphasized that this formula will produce an indefinite amount of soil sterilization. Its chloride fraction is intended to reduce the hazard of fire in weeds which have been destroyed by its action.

No. 1	$\left\{ \begin{array}{l} \text{Sodium chlorate—100 pounds} \\ \text{Calcium chloride—100 pounds} \\ \text{Add water to make a total volume of 100 gallons.} \\ \text{Stir until dissolved. Cost: $14.00 per 100 gallons.} \end{array} \right.$
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#### *For Roadsides, Permanent Ditches, Stone Hedges, etc.:*

If it is desired to destroy weeds in areas not agriculturally extensive, i.e., not

in open fields, and where partial soil sterilization would be immaterial or even an advantage, the following formula may be used. Furthermore, if the area to be treated could not be handled by implements, such as a stone hedge, and if the increased cost of materials would not be prohibitive because of the relatively smaller extent of the specially situated weed infestation to be destroyed, then this same formula we believe will be found highly satisfactory and withal considerably cheaper than the standard chlorate formula mentioned.

No. 2	<table border="0"> <tr> <td>Sodium chlorate—50 pounds</td></tr> <tr> <td>H.S.P.A. Activator—3 pounds</td></tr> <tr> <td>Dissolve in water to a total volume of 100 gallons.</td></tr> <tr> <td>Cost: \$4.60 per 100 gallons.</td></tr> </table>	Sodium chlorate—50 pounds	H.S.P.A. Activator—3 pounds	Dissolve in water to a total volume of 100 gallons.	Cost: \$4.60 per 100 gallons.
Sodium chlorate—50 pounds					
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Dissolve in water to a total volume of 100 gallons.					
Cost: \$4.60 per 100 gallons.					

*Directions:* Apply as a spray to destroy weeds. Ten days later, provided a temporary sterilization of the soil is desired, remove all surface litter and apply again as a drench upon the soil or in crevices where weeds formerly grew.

#### *For Perennial Grasses Difficult to Control:*

To destroy grasses and weeds in ditches within the field or on borders of fields or in other localities where it is essential not to sterilize the soil or injure the current crop through its root systems, the following formula, as a rule, may be used to advantage. Before applying this formula on a large scale it is important to determine the tolerance of crop and soil to this particular solution in a number of small test trials.

No. 3	<table border="0"> <tr> <td>Sodium chlorate—20 pounds</td></tr> <tr> <td>H.S.P.A. Activator—4 pounds</td></tr> <tr> <td>Dissolve in water to a total volume of 100 gallons.</td></tr> <tr> <td>Cost: \$2.40 per 100 gallons.</td></tr> </table>	Sodium chlorate—20 pounds	H.S.P.A. Activator—4 pounds	Dissolve in water to a total volume of 100 gallons.	Cost: \$2.40 per 100 gallons.
Sodium chlorate—20 pounds					
H.S.P.A. Activator—4 pounds					
Dissolve in water to a total volume of 100 gallons.					
Cost: \$2.40 per 100 gallons.					

This solution is very effective on above-ground portions of mixed-weed populations. Since it exercises little if any sterilization effect on soil, crop or weed root systems of perennials, a repeat application will have to be made as usual in due course. It should be applied as a spray to weeds only and *not* as a drench on the soil.

#### *For Ordinary Mixed-Weed Growth in Which Annuals or Succulents Predominate:*

The following formula, applied as a spray by machine or hand-operated implements, appears to give satisfactory results for large-scale field treatment. It is not to be classed as an ultra powerful herbicide, but for general purposes it is perhaps one of the most advantageous weed killing solutions to be obtained.

Its advantages may be stated thus:

1. It is non-poisonous in the sense that it produces no apparent external ill effects on the spray operator. It is equally advantageous in discouraging grazing animals from eating freshly sprayed vegetation because of the objectionable taste of the highly diluted activator it contains.

2. Because of its low concentration of chlorate and due to the fact that a large proportion of its chlorate fraction appears to be reduced to simple table salt in its action on weeds, its fire hazard, it seems, is no more severe than may obtain

similarly in any other herbicidal treatment where large areas of dried weeds are left standing in the field.

3. It does not sterilize the soil—as far as we have been able to determine.
4. It does not decompose rubber or attack metal spraying equipment during normal service usage.
5. It does not appear to require the addition of extra wetting or sticking agents.
6. It may be prepared from dry chemicals which do not decompose on exposure and may be dissolved at any convenient station, in or out of the field, where a supply of water is available.
7. It is considerably cheaper to prepare than any chlorate-chloride combination having comparable weed killing properties and, incidentally, any such chlorate-chloride solution of equal field value appears to occasion chlorosis in the crop and to partially sterilize the soil.

The formula:

No. 4	Sodium chlorate—15 pounds
	H.S.P.A. Activator—3 pounds
	Dissolve in water to a total volume of 100 gallons.
	Cost: \$1.80 per 100 gallons.

*Directions:* Apply as a spray, using from 50 gallons to 500 gallons per acre, depending on the stem and leaf area and density of weeds. (Occasional weed patches between cane lines may use but 50 gallons per acre. An open field of solid weeds, waist to shoulder high, would require about 500 gallons per acre.) The formula may be strengthened for treatment of tough or persistent grasses by increasing the activator to 4 pounds per 100 gallons.

#### *For Guava, Lantana and Other Woody Pests in Pastures:*

Use formula No. 4 as a heavy drench on the individual stool applied from a sprinkling (not a spraying) device at the base of the plant and not on the foliage. Plants having extensive root runners should be pulled out a few months after treatment. This feat may be accomplished with ease, it has been found. A repeat drenching may be required, however.

#### THE ENCROACHMENT OF PERENNIAL TOUGH, WIRY GRASSES UPON AREAS CURRENTLY DENUDED OF SUCCULENTS OR BROAD-LEAF ANNUALS

This discouraging development is due entirely to the regulation technic of weed spraying as it is generally used. An overall spraying of mixed field weeds destroys all the annuals and succulents it contacts and, in addition, the above-ground portions of tough, wiry grasses. In about 2 weeks new growth appears in the grass patches, whereas the annuals and succulents are out of the picture. With surprising vigor and speed the grasses will now spread to and take possession of the denuded areas formerly occupied by the annuals. In a few seasons the field will be covered with the more persistent and in many cases high growing and dense grasses.

It has been our experience in situations such as these that the only practical

spraying treatment which will arrest the grass encroachment is to respray new grass growth for several successive intervals about two weeks apart. This procedure is not a practical one except for open places of relatively small area. Within a cane field up to the closing-in period the method may be used to repress grass over other weed growth.

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## Further Studies in Nitrogen Nutrition

### AMOUNTS-OF-NITROGEN TEST

By A. H. CORNELISON and H. F. COOPER

In *The Hawaiian Planters' Record*, 40:35-56, 1936, a report was published on "The Effect of Nitrogen on Cane Yield and Juice Quality" by U. K. Das and A. H. Cornelison. The report covered a test of a preliminary nature to establish certain fundamental bases from which could be launched a set of more comprehensive attacks on the effects of nitrogen on the cane plant.

With the information gained in that first study, three new tests were laid out and carried to completion. One test approached the problem from the angle of the amounts of nitrogen applied, the second, from the angle of the time these amounts were applied, and the third, from the angle of the effect of water on optimum nitrogen-fertilized plants to be grown at Makiki.

The methods of studying the effects of these treatments fall into the fields of biochemistry and plant physiology and would be of no interest to the fieldman. For this reason the methods will not be treated in detail here, but to those who are interested voluminous data are available in the technical reports (Project D-1) which are on file at the Experiment Station. The discussion presented hereafter will be concerned chiefly with the practical results of the tests. While these three tests are to be discussed individually, they may in turn be considered as components of one large test when results of the various treatments are considered. For this reason, in the final report a general discussion and summary of interlocking information will be included.

The field plots were all uniform in size and general growing conditions as well as in their original stand of cane. Each plot consisted of 20 lines, 20 feet long by 5 feet wide, and was separated from adjacent plots by six-foot trails. The original stand of cane was initiated at 65 primary stalks per line to furnish fully comparable results on mortality and tillering when each line was harvested. These stalks were all tagged and called "first-order" canes; they made up the greater portion of the crop.

At harvest the canes were divided into different sections which were determined by the season and age during which such parts of the stalk were formed. This sectioning of the stick was done so as to determine the amount of cane formed, as well as other characteristics that might have been caused by age and season of the year or weather. The section formed in the first four months was called the "four month's" section, the second four month's growth was called the "eight month's" section, and the second year's growth was divided into mature "dry-leaf" cane, "green-leaf" cane, and "non-millable top."

Starting at the age of two months and every two months thereafter, one line of cane was harvested from each plot and the necessary data collected. Thus we have almost a complete life history of over seventeen thousand sticks of "first-

order" canes and less complete information on many thousands of other sticks which came up in the second-year growth period.

As each test was subject to a specific fertilizer treatment, the methods will be described as each of the tests involved in the experiment is discussed.

#### AMOUNTS-OF-NITROGEN TEST

This test comprised four plots, each of which received 200 pounds of potash and phosphate per acre in one dose, when the cane was one and one-half months of age. At this same age all plots received their differential nitrogen treatments in a single application. The "A" plot received no nitrogen, the "B" plot received 100 pounds per acre, the "C" plot 200 pounds per acre, and the "D" plot 400 pounds per acre. Irrigation water was applied in 2-inch bi-weekly doses or if rain intervened the supply was adjusted to give 16 inches per month to all plots alike.

##### *Length Growth (Figs. 1, 2, 3, 4):*

To determine the relative effects of the treatment differences upon the cane growth, 25 stalks in an inside line of cane were selected in each plot for monthly measurements. These growth measurements, as in a former test, clearly exhibit the increase in length of stick from the higher nitrogen treatments. Once an optimum amount is reached, however, no great increase in growth is made by additional nitrogen applications. The rates of elongation in this test, as formerly, show a high correlation with temperature, and point to the seasonal variation in temperature as the limiting factor in the vegetative growth of the cane plant. Seasonal variation in temperature, therefore, limits the cane tonnages obtained in Hawaii.

The data accumulated in this series of test plots indicate that at or slightly above an average day and night temperature of 67° F. the rate of cane growth is constant, regardless of the nitrogen fertilizer treatments. The response in growth rate which is the result of temperature increases above 67° F. appears to be immediate, at least within the measurement interval of one month.

The data also reveal that measurements of stalk elongation do not give a direct picture of cane weights, especially in the plots under excessive nitrogen treatments, for in this case the sticks, although longer, are lighter. In addition to this discrepancy it is evident that the present procedure employed in measuring stalk elongation, although the best now available, yields results which are at most only indicative of the growth which actually occurs. The elongation data are in error from season to season because the apex or terminal bud is not in a position of constant relationship to the last appearing dewlap.

##### *Suckers (Fig. 5):*

An accurate record of all secondary growth was kept and considerable information on the effects of nitrogen was obtained therefrom. The 65 original sticks made up close to 70 per cent of the total cane harvested up to the twentieth month. The 30 per cent of secondary sticks comprising the remainder of the crop for this period consisted of cane which started in the fall of the first year, some four months after the primaries started. The secondaries or suckers which sprouted in the

second season were not completely mature in twenty months of normal crop life and, in the case of H 109 cane, can perhaps be disregarded. (In other canes, such as POJ 2878, the second-season canes do become highly important and make up a major portion of the crop, if not the entire crop, at twenty-four months.)

The application of nitrogen in increasing amounts correspondingly encourages these second-season canes but, as will be brought out in a subsequent paper on the "Time of Application," the use of nitrogen fertilizer in multiple doses greatly increases this effect.

*Mortality of Cane (Fig. 6):*

Death claimed a considerable number of sticks in the higher nitrogen plots and this mortality increased with age. Some of the stalks died from the effects of competition and crowding but a large number died for no apparent reason and while still in fair growing condition, especially in the two high-nitrogen treatments. While a poor explanation, this would seem analogous to some human beings—"to have lived a little too fast in youth" and had to pay for it later. Results from a variety test which was grown concurrently with this nitrogen study show that varietal inheritance plays a great part in this death rate. For instance, at 24 months of age POJ 2878 had not a single first-year stick left alive—the entire stand was secondary growth.

In our 1936 test we found increased incidence of borer damage in proportion to nitrogen applied and blamed this for the high stalk mortality. It becomes evident, however, that the cause is within the plant itself, as the cane disclosed no borer infestation in this test.

While we cannot put our finger on exact causes for the death of many stalks, we can attribute it to two really opposed sets of conditions under one and the same fertilizer treatment. Early in the life of the crop, mortality is due chiefly to some form of starvation (plant food, light, water), whereas, later on in the development of the crop, the very sticks that have induced starvation in the first group die from their excesses. It must be remembered too that cane shoots do not all start out free and equal with the same constitutional vigor, and some are left by the wayside by their stronger brothers.

*Yield of Cane (Fig. 7):*

For purposes of investigation the cane as harvested was divided into sections, determined by the season when such sections were formed, as well as the age and maturity of these parts at each harvest. Thus it was possible to trace the life history and characteristics of the canes under many conditions.

Top-to-stalk ratios for three definite periods in the life of the plant were discovered which held their identity regardless of fertilizer treatment, and which appeared to be a varietal characteristic: (1) during the first year, the ratio of tops to stalk decreased rapidly; (2) in a second period which occurred during the winter of the first year there was a less rapid decrease in this top-to-stalk ratio; while (3) in the third period which began during the winter of the second year and held to the end of the crop, the top-to-stalk ratio was constant at about 12 per cent.

Probably of greatest interest is the fact that these ratios indicate that there is, at any age of the cane plant, a fairly definite top size or mass for a given weight

of stick. At maturity the weight of top was 12 to 15 per cent of the weight of the cane under the conditions of these tests.

Nitrogen applications have increased the weight of leaves, so it is reasonable to suppose that nitrogen fertilization first builds heavier tops and that these larger tops are then responsible for increased sugars which are used in building more tissue for storing sugar and increasing "tonnage."

Season of the year and temperatures control top formation, while increasing age tends to diminish top activity. In spite of treatment a decrease in leaf size and change of leaf shape occurs with age. As a direct result, therefore, the size and weight of stick being formed is also decreased. Confronted by these factors it becomes obvious that we should give the plant its nitrogen fertilizer early in its growth period if we are to expect greatest efficiency per unit amount applied, although by so doing we may increase mortality of much of the original stand of cane at about 16 to 18 months of age.

When season of the year interferes with early application, the efficiency per unit amount of nitrogen applied will be lower as far as cane tonnage is concerned and it appears from our data\* that ripening will be incomplete for a longer time.

#### *Juice Quality (Figs. 8, 9):*

When we speak of juice quality we refer to the quantity of sucrose in a given quantity of expressed cane juice. Not only is the sucrose in this juice considered, but also other materials which are present in the sap, such as salts of various kinds, and sugars other than sucrose as well as a multitude of other compounds which are removed as filter cake in the mill. The weight of sucrose expressed as per cent of the total dissolved solids in a juice is known as its "purity." Its purity, subtracted from 100, tells us the amount of these undesirable solutes which are present as percentage of the total dissolved material in the juice.

If two cane plants contain the same amount of sugar but one has more water in it than the other, the wetter stick yields poorer juices. Again, if one plant has more salts than the other, the saltier stalk yields poorer juices.

The quality of the expressed juice is designated by its per cent sucrose and its purity. By carrying juice quality one step further we have the term "quality ratio" which, by definition is: (1) a formula using the two above juice quality figures, (2) a factor for the relationship of per cent sucrose in cane to sucrose in juice, and (3) a fixed set of manufacturing constants that indicates the number of pounds of cane to make one pound of commercial sugar.

As is commonly known, heavily nitrogen-fertilized cane usually gives poor juices. Naturally the question arises, was there really less sucrose in these plants or was the effect due to water in the plant being unduly increased and thus diluting the juice on expression in the mill.

#### *Water Content of Plants (Figs. 10, 11):*

The problem thus resolves itself into a study of the water, sugar and ash relationships in the plant at various ages. From our former test we know that season

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\* The data referred to will be discussed in a paper to follow entitled "Time of Application of Nitrogen."

of year, temperature, and age caused the largest variation in these constituents and, therefore, we again followed these effects closely.

Careful study of the data shows that if nitrogen is present in excessive amounts in the soil during the early growing period of the plant, the water content of the tissue formed under this set of conditions is proportional to the nitrogen application and the effects so induced are never entirely eliminated by ripening or age.

This watery, high-nitrogen tissue also behaves differently in the mill than does the tissue from more normally grown canes. Our data indicate that we can remove more water proportionately by pressure from high-nitrogen cane than we can from low-nitrogen cane. Consequently, the juice from high-nitrogen cane is diluted more than a comparison of the true water contents of the two plants would indicate. As the nitrogen application is increased, the additional water expressed from the tissue seems to carry with it impurities which render the juices poorer in quality.

In a former paper this increased water content was attributed to an increase in salt content of the higher-nitrogen plants. Data from this present test, however, reveal that the per cent of salts or ash in the cane is really reduced in the case of higher nitrogen application. Only one element, calcium, seemed to increase in the plant under increasing nitrogen treatments. This alkaline earth is known to have the property of swelling cellulose and allowing it to absorb water, and part of the increased water in the plant may be held in this condition.

While we have been unable to prove that the increased impurities in the juices, such as acids and electrolytes of one kind or another, are also in some way associated with the water content, we strongly suspect them of playing a part in this complex.

As the crop makes growth during the first winter, this greater water content is also found in such growth but, unlike the first summer's excessive nitrogen tissue, this winter cane material does lose its water with age and ripening, so that by harvest time it is quite similar in both the high-nitrogen and the low-nitrogen plots. So, too, does the water in the second-season growth appear to be influenced by the nitrogen applied during the first season, but it also comes to a common water content under all nitrogen treatments, if it is given time. Thus it may be said that tissues formed under conditions of high or excess available nitrogen, in good growth weather, are more or less permanently impaired for sugar storage, but tissues formed later when this nitrogen supply is reduced suffer no permanent impairment.

In the former paper we gave the impression that this increased water content caused the major upset in the plant cells and the poorer quality of juices. Today we do not feel as free to say that the water is the cause of these changes in sugar content. It now resembles the old argument as to which came first, the hen or the egg. We do know that whenever growth is accelerated the sucrose percentage is lowered and water content is raised. Nitrogen compounds will accelerate this growth under proper conditions. Thus it is purely conjectural to blame the water entirely. We do know that water content can influence the reactions which are catalyzed by the enzymes in a plant cell, but primarily some factor must have induced the water to be present in the cell to set up the train of events that followed, and it is this, factor or factors, that we must isolate. Hence we are unable

to state whether the sugar is decreased first and the high water content resulted, or vice versa.

The seasonal fluctuations in the water content of the mature sections of the stick give us a picture of the transpiration flow of the plant and also of the water which is absorbed into the cells of this tissue under variable weather conditions. The increased cell hydration apparently influences the sugar content as this increase is closely followed by increases in reducing sugars which are presumably used in the respiration processes of the cell.

*Sucrose Content (Figs. 12, 13, 14, 15, 16):*

As in the former test, we have determined the sucrose content of the cane in two ways: first, by crushing the entire harvested line of cane in a Cuba mill and analyzing the expressed juice, and second, by securing the total sucrose in the cane stalks on their oven-dry basis.

As was to be expected the sucrose in the expressed juices was lower in all parts of the plant in proportion to the increased nitrogen applied, up to the age of about twenty months, but parts of the crop which were formed after this age lost this relationship. Season of the year has a great influence on the sucrose in the juice but this is also modified by the age of the plant. In the cooler months of the spring (February, March, and part of April) juices reach their best concentrations. Increasing age of the cane, however, appears to steadily improve these concentrations so that where full genetic maturity and season coincide we achieve the optimum sucrose concentration in the juice. One fact that readily impresses itself regarding sucrose storage in the plant is that cane tissue which is formed under the stimulus of high nitrogen is never able to carry as much sucrose per volume of juice as is cane tissue formed where this excess nitrogen supply has been reduced.

Total pounds of sucrose present in the cane of the two higher nitrogen treatments at each harvest were about the same, so the poorer sucrose concentration in the juices of our high-nitrogen cane was a case of the diluting effect of water in what appears to be permanently more succulent tissue. This succulence naturally raised the number of pounds of green cane required to produce a pound of sugar. The question naturally arises here, could the high-nitrogen canes produce as much sucrose as the lower-nitrogen canes, and if not, why not? We found that whenever the water content of the cane was high the reducing sugars were also increased in the plant, while, at the same time, sucrose was reduced, but that the reduction in sucrose at any time was always greater than the increase in reducing sugars, and the books would not balance. An attempt was made to determine whether sugars were being changed to so-called fiber or cell wall material but from the data at hand it seems that once the cane had reached the dry-leaf stage of life, only traces of such a conversion occurs, if any at all.

With the reasoning that all living cells need energy to maintain life, the picture then resolved itself into one in which the cells were utilizing the sucrose for fuel and the reducing sugars which we detected were sucrose fragments, either in the process of being burned in the cells or on the way to the formation of other cell materials to replace those destroyed and eliminated with age. The continuous increase of the organic acids in the plants as they aged also pointed to a partial or incomplete burning of sugars for maintenance of life or a break down of cell

material of carbohydrate nature. Thus in the presence of higher nitrogen supply and good growing temperatures the cells split up their stored materials more rapidly and it was possible to detect greater amounts of reducing sugars at harvests when the activity was great. Thus with increased age and decreased cell activity reducing sugars can be expected to be present in less and less quantities but never completely absent as long as the cane is alive.

As nitrogen is the important element in the formation of proteins which go to make up the living substance or protoplasm in a cell, and since in this protoplasm complex the life processes of the cell are performed in the presence of relatively large amounts of water, a part of the water increase and sucrose decrease could arise from an increase in the protoplasm or its activity under high nitrogen fertilization. The above conception of the plant metabolism furnished no help as to the efficiency of the nitrogen-fed plants as sugar producers but did give some idea of their possible destructive efficiency.

The fact that the leaves of the plant are its sugar factory led us to set up ratios of the total pounds of sucrose present in a line of cane to the weight of leaves in that line at the different harvests. Oddly enough the ratios for efficiency of normally (not nitrogen starved) growing canes were the same at any age when the nitrogen was applied in one dose early in the life of the plant. This rather definitely pointed to the fact that the per-pound green weight of leaves under different nitrogen treatments could produce about the same amounts of stored sucrose, and leads to the general conclusion that the higher nitrogen canes were as efficient sugar producers as were the lower nitrogen canes (but not nitrogen-starved cane). The differential seasonal concentrations of sucrose found in the juice and in the total dry-weight must then be due to differential utilization in the cells or some condition of the cells themselves from season to season.

Since vegetative growth, as well as respiration or life activity, is greatly influenced by temperature and can thus be regarded as thermo-chemical in behavior, and is promoted by nitrogen, it becomes necessary to continually replenish the sugars which are being used up in the respiration. Photosynthesis or sugar manufacture is not as critically affected by temperature as are growth and respiration. Thus with the onset of the lower seasonal temperatures and their resulting inhibition of both growth and respiration rates, the larger tops in the higher nitrogen fertilized plots can produce more sugar for storage than the lower nitrogen plots.

Simply put, the relationship is this: during higher temperature and under high nitrogen supply, respiration losses plus the sugar used to form cellulose and lignin and the other cell wall materials needed in growth, tend to leave reduced amounts for storage in the newly formed cells and less for replacement of losses in old cells; but with the change of season to cooler temperatures the plant backfills the storage cells, water is reduced in volume, and juices are improved.

#### *Glucose (Figs. 17, 18, 19, 20):*

The presence of glucose, or more correctly reducing sugars, in the plant was considered, in the former paper, as inversely related to the sucrose content. In the tests in this experiment we find the same type of data for the seasonal and age variations of this constituent.

The relationship of reducing sugars to the water content of the plant is also

parallel to the former test. However, from micro-chemical data and results from the present tests a new conception as to the role of this reducing sugar has developed.

In the previous tests we felt that losses in sucrose were to a large extent represented as increases in glucose or in the hemicelluloses found in the plant. The conception arrived at in these tests is that reducing sugars which are present at any time in amounts above one-half of one per cent in the older parts of the plant are indicative of cell activity of a catabolic nature and can be used as a rough measure of maturity of a given crop.

The reducing sugars fraction in a plant according to our latest conception is thus to be considered in two major roles; first, fuel for cell respiration, and second, raw material for cell construction. The first classification is chiefly effective in older plant parts, and the second in the young growing parts or at meristematic centers.

Decreases in sucrose content of tissue are never balanced by increases in reducing sugars or increases in dry matter in corresponding tissues. Thus we must assume rather rapid translocation or consumption by cell respiratory functions. Since the decreases in sucrose are generally similar throughout the entire plant at any time, the evidence all points chiefly to rapid consumption by respiratory processes rather than movement in the plant. As the respiratory activity occurs chiefly in nitrogenous materials in the cell, applications of nitrogen and the resultant increases in nitrogen in a cell tend to increase respiration rates. The respiration rates, however, are heavily influenced by temperature and vary directly with it. The nitrogen in the cells is gradually reduced from the time of youth to maturity and respiration rates drop correspondingly and appear to approach a constant value in old tissue for a given season.

In general, micro-chemical work on conducting tissue, as well as analytical data for anatomically different parts in a complete stalk joint, lead us to believe that glucose (or more generally reducing sugars) is not the normal translocatory form of carbohydrate in the cane plant but rather that the sucrose is the normal movable form, as well as the normal storage form.

Other data we have collected definitely show that there exists varietal characteristics as to reducing sugars in the plant which even at maturity do not reach a common level—H 109 cane is in general always higher in its content of reducing sugars than either 31-1389 or POJ 2878 at maturity.

#### *Pectic Substances (Figs. 21, 22):*

In the former test we analyzed the cell material for easily split carbohydrates which were called polysaccharides, but we gained little from the study as the data from the determinations were inconclusive. Methods of studying the chemistry of most cell wall materials are quite arbitrary. However, in the case of one cell wall material, the uronic acids, the determination is satisfactory, and we have studied these compounds. In general they are made up of three classes—pectin, proto-pectin, and pectic acid—all of which have the same basis of sugar-acid structure. (Pectin is the common jelly-forming material in fruits that make possible formation of fruit and sugar jellies for the table.) In the plant cell wall they are found in the form of metallic salts, pure pectin, and a forerunner of these

two. The middle layer or lamella between the cell walls is largely pure pectin. To a large extent, the salts of calcium and pectic acid in the cane plant lie in small granules in the cellulose inner layers of the cell wall. Some cell structures such as the phloem tissue in the cane carry proportionately heavy amounts of pectic-class materials. Under proper conditions all may be made water soluble and they can be thus considered as part of the materials involved in expressed juice purity, as well as playing a fairly important part in clarification problems in the mill.

Our studies on these materials indicate that the percentages are high in the young cane tissue and decrease in the older tissues to about a constant value of two per cent on the dry-weight basis. However, no effect of the nitrogen treatments was found in the data concerning these pectic substances. The age of the plant has the most important effect on the content of these uronic or pectic compounds.

The micro-chemical data which we collected on tissues of various ages lead us to believe that the differences in the percentage of pectic materials between the young and old parts of the cane are more apparent than real because of the percentage increases in other cell materials that occur with increasing age. However, at one period in the life of the plant, we are uncertain of the veracity of the above statement, as very late in the life of the plant we found an increase in the content of these materials which at present we cannot explain satisfactorily. Physiologically the change may be of importance and a repeat of these determinations should be carried out as the change occurred about tasselling time in mature cane.

We feel that the calcium salts of pectic acids may be a factor in the water content of cane tissue, especially in the cellulose fractions of the cell wall.

#### *Nitrogen Content of the Cane (Figs. 23, 24, 25, 26):*

As we applied various amounts of nitrogen to the different plots it was only reasonable to expect to find nitrogen in the plant in rough proportion to the treatments applied. In this test we determined the soluble nitrogen fractions giving the Van Slyke amino acid reaction in the Cuba mill expressed juice. While not strictly correct, chemically, we called these "amino acids" and considered them to be soluble non-protein nitrogen (or non-living forms of nitrogen) that were moving or could be moved in the plant.

The total nitrogen figures show the heavy uptake of nitrogen immediately after its application. All parts of the plant respond but the foliar tissues and tissues adjacent thereto show the greatest fluctuations.

Starting with the lower end of the cane stick we find total nitrogen percentages decreasing with increasing age. The entire stick, above the butt to the lowest attached green leaf, reveals this diminution steadily with age until a percentage of about .10 on the dry-matter basis is reached, at which figure the various parts tend to become constant.

Above the lowest attached green leaf, tissues were found to be lower in total nitrogen percentage with age but there was more nitrogen in this section than in tissue where leaves had dropped. Seasonal influences in the trends of the nitrogen curves for both dry-leaf and green-leaf sections are present but the age influence is by far more obvious.

However, as we study the non-millable top section we find that the total

nitrogen percentage in the top, being low in summer and high in winter, reflects the effect of season. This effect is largely due to the relative growth rates at these two periods and the speed of movement of nitrogen from the lower parts of the stick as it ages. We find the data on the alpha amino acids in the top juices paralleling the total nitrogen figures seasonally but in addition we find that in summer when less of these compounds are detected in the top sections a greater amount is recovered in the lower parts of the stick. The picture appears to be one in which the summer season speeds up the rate of movement of soluble nitrogenous materials toward the top while the winter season depresses this movement. It is impossible to say from our data, or lack of data as far as roots are concerned, in what form this nitrogen existed prior to its release into soluble form.

We did some micro-chemical work on the cane stick and found nitrogenous material stored or lodged in the tissues of the node immediately under axillary buds, root primordia, and to a lesser extent in the protoplasm of the storage cells. With increasing age, the nitrogen material in the node complex appeared to diminish to a larger extent than in the internode and it is suggested that this locus is the one in which the greater portion of the nitrogen in the stalks of the plant is temporarily stored.

However, from our micro-chemical work, it becomes quite probable that nitrogen may be fed to the apex from a multitude of other locations: from older leaves, from cells in the fibrovascular system as they lose their protoplasm, and from nodal tissue and sugar storage cells as they approach complete maturity. We feel that roots also play a part in storage but have no data on this portion of the plant. The analytical data all point to one fact of great importance and that is that the apex or growing top of the stick exercises a preferential demand or occupies a preferential position as far as water and nutrient supply are concerned. Under shortage of water or nutrient materials the remainder of the stalk below the top will supply the lacking material to the extent of its ability. This condition sets up two practicable indices for the fieldman: nitrogen in leaf-punch samples, and slowing down in movement of leaf triangle, in the accepted growth measurement method, under water shortage conditions.

The nitrogen in leaf-punch samples from young leaves remains indicative of high nitrogen levels in the top after the levels in older parts of the stick have been pulled down by the apex demands. Thus a lowering in nitrogen content in these leaf samples comes after supplies in other parts of the plant are reduced to a point below which possible growth losses may take place unless additional nitrogen is supplied.

The effect of growth retardation of the last exposed dewlap or leaf triangle, however, will be felt before the apex itself is detrimentally influenced and the fieldman can have some latitude as to time of application of water after the symptoms occur. From available data, however, the duration between the time leaf sheath growth is effected until apex growth is affected cannot be properly ascertained. Naturally, the period will be variable due to age of cane and season of year.

The nitrogen leaf-punch figures should also be of value as an indicator of maturity since a low percentage of nitrogen will indicate a cessation of growth in a short period of time thereafter. All our data point to the need for the

application of the total nitrogen within six to eight months of age. If the nitrogen supply is inadequate before this period is completed, judicious application especially in long crops will be advisable; but if a normal cane crop is over ten months of age it is most strongly recommended that no further applications be made as nitrogen applied after this age is relatively inefficient and the results may be disastrous to the juice quality.

*Summary:*

In summarizing the results of this test, we are attempting to draw a picture of the metabolic behavior of the plant as a whole rather than the usual stressing of the individual, detailed determinations.

Higher water content, reducing sugar content, variable mineral content, and the detailed field weights are linked together to show that they are only manifestations of conditions induced in the plant by the cooperative effects of the nitrogen fertilization, genetic age, and weather.

The effects of nitrogen fertilization on the sugar cane plant are largely controlled by the weather conditions existent during the time of most active utilization of nitrogen by the plant. Of the effective factors making up the weather complex, temperature, above a certain minimum, plays the most important role, through the acceleration of vegetative activity and respiration rates. The genetic age of the plant influences the magnitude of the temperature effects and likewise the sucrose storage at any time. The physical and chemical properties of the cell structures are greatly influenced by variable rates of vegetative activity and, in some cases, the changes induced are apparently more or less permanent.

The difference between sugars elaborated by photosynthesis and the fractions used in tissue formation and respiration determines at any time the amount of stored sucrose. Nitrogen applications appear to produce increases in rates of all these functions up to a certain limit, beyond which optimum application there is a disproportionate increase in the tissue formation and respiration rates and a resultant lowering in sucrose storage per volume of stalk. The optimum application thus becomes dependent upon temperature or age conditions as to magnitude.

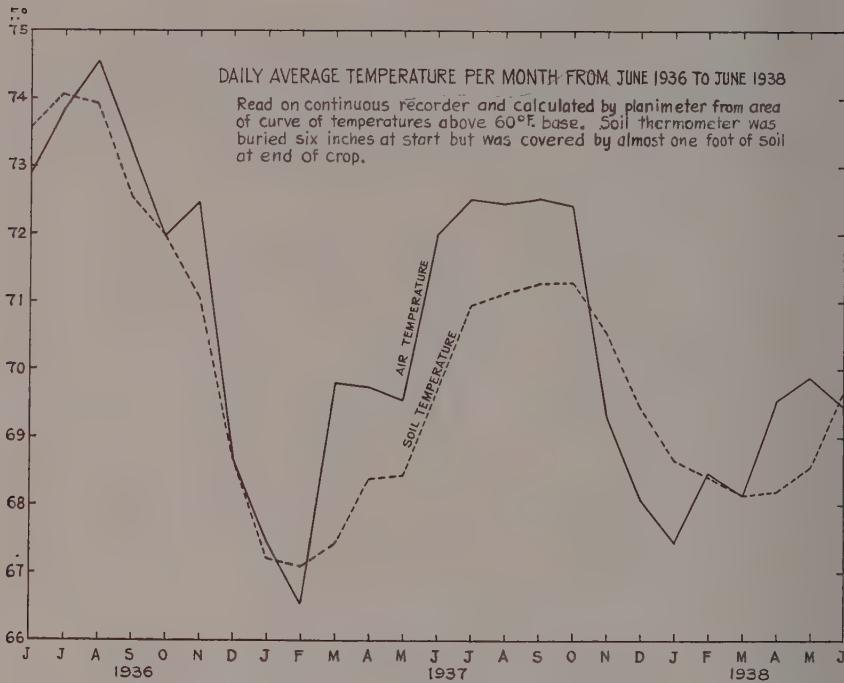


Fig. 1

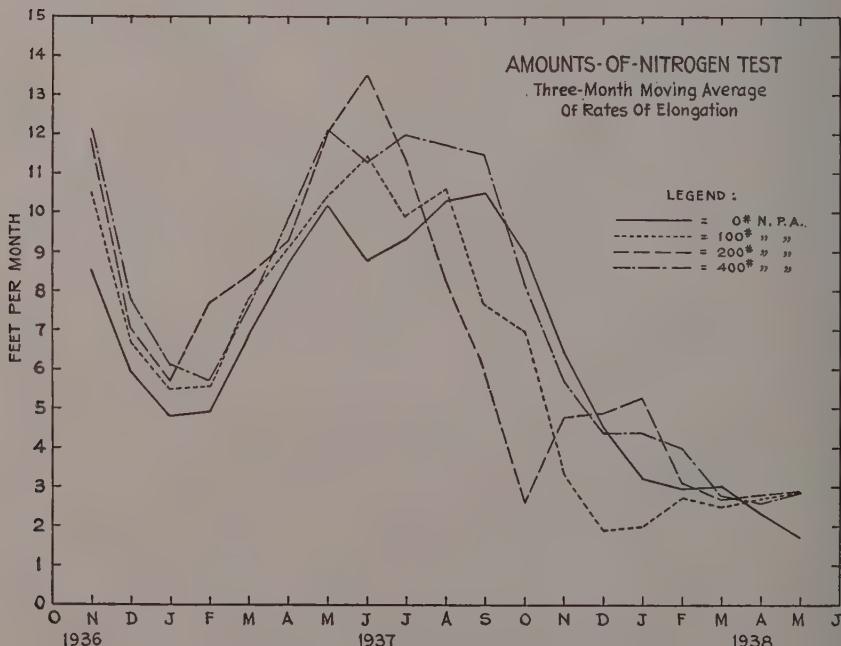


Fig. 2

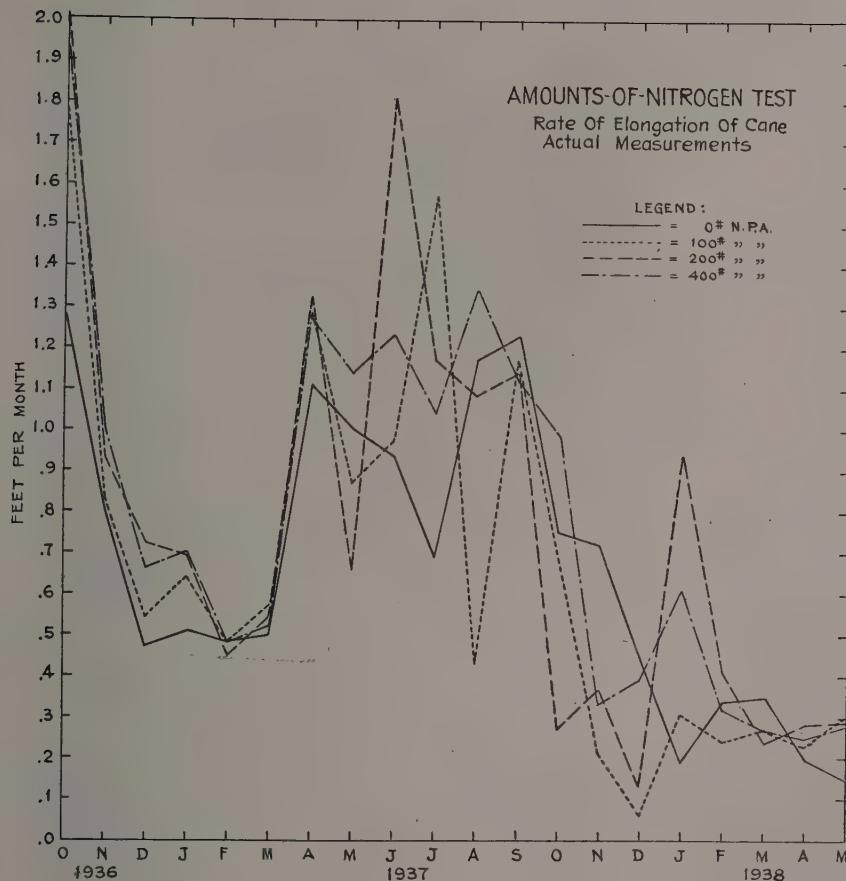


Fig. 3

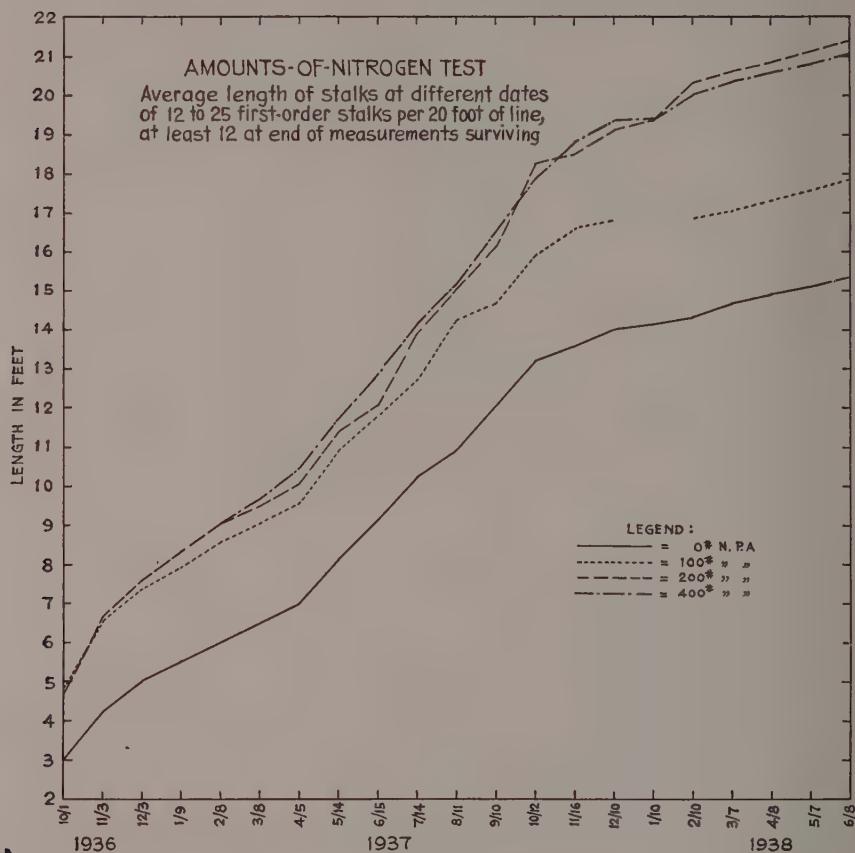


Fig. 4

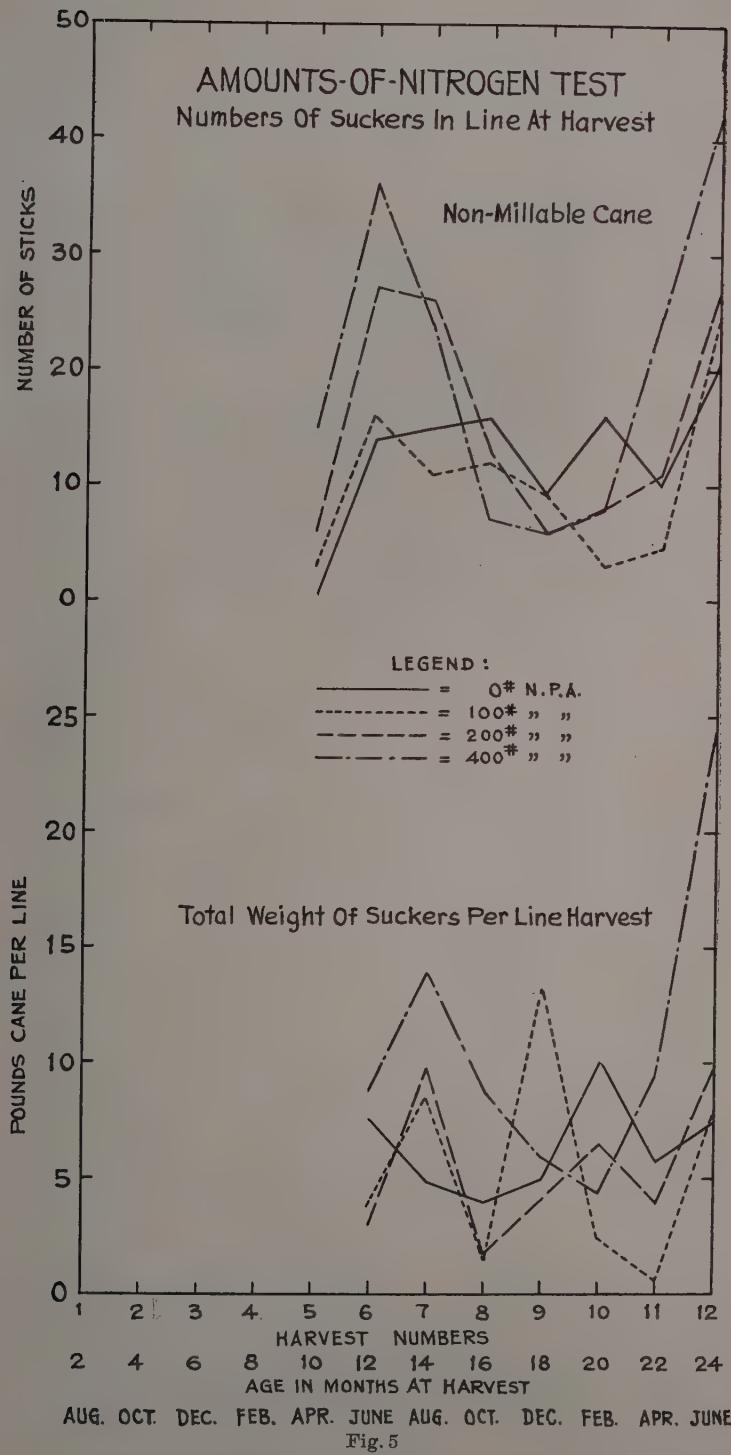


Fig. 5

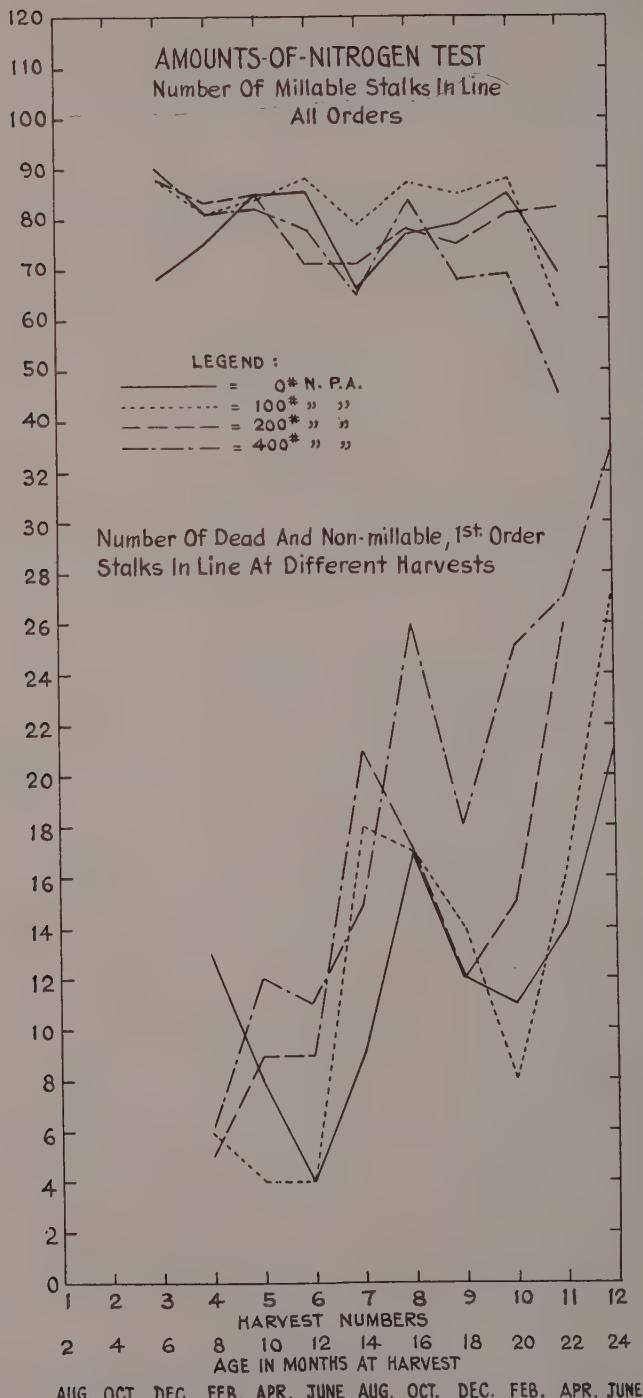


Fig. 6

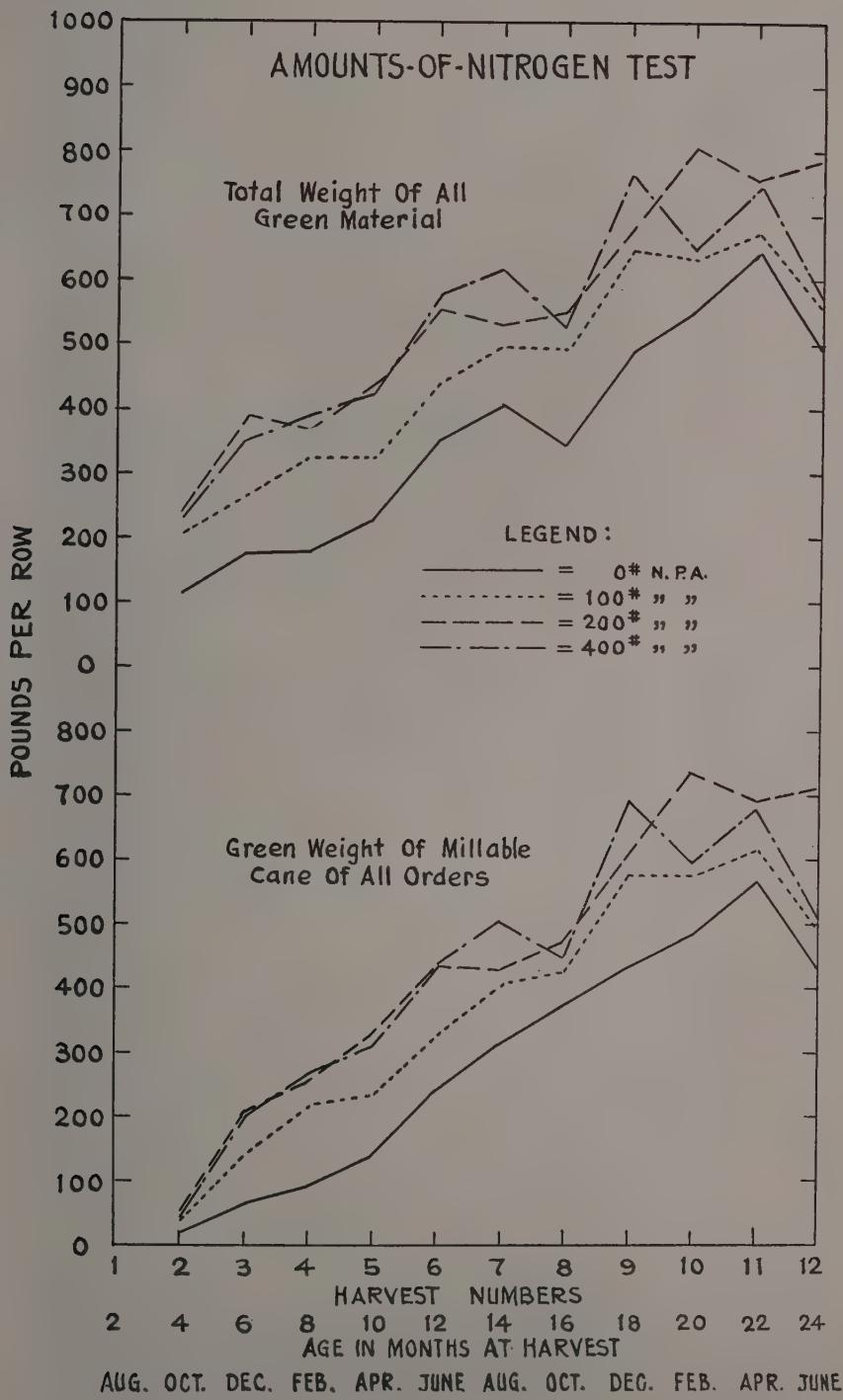


Fig. 7

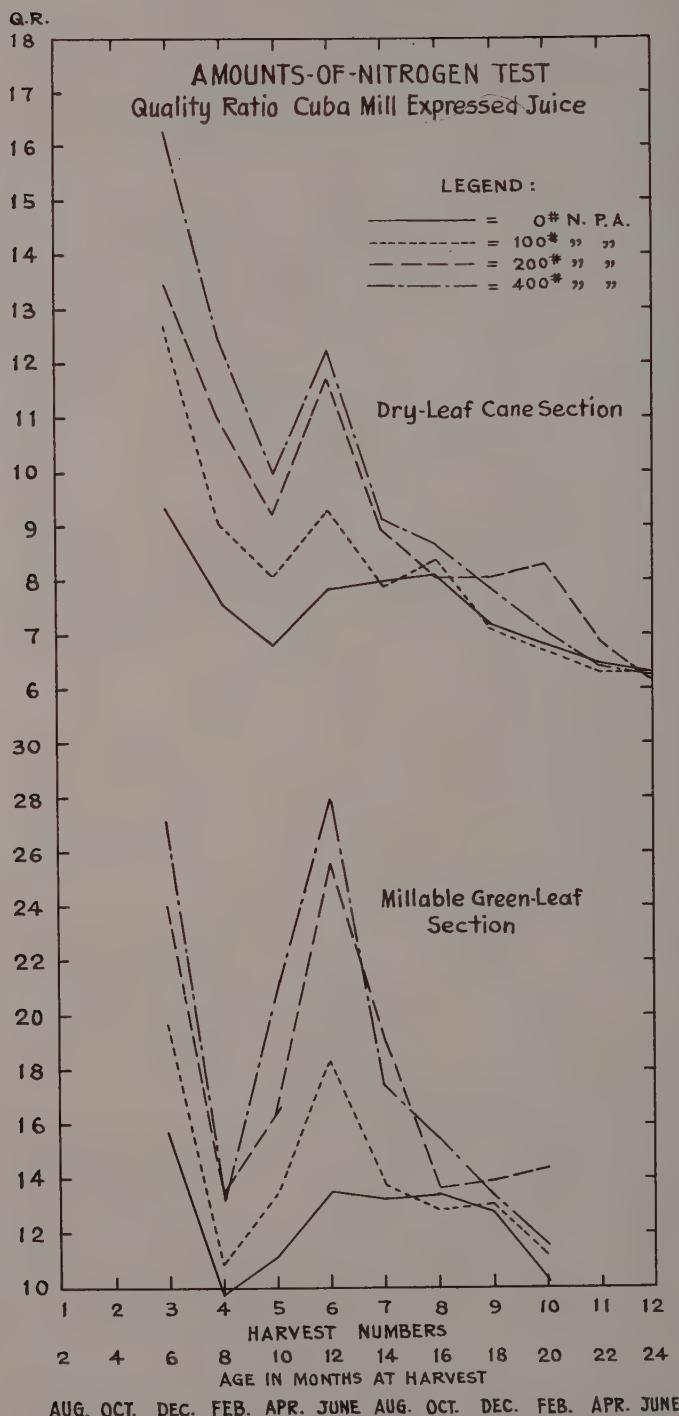


Fig. 8

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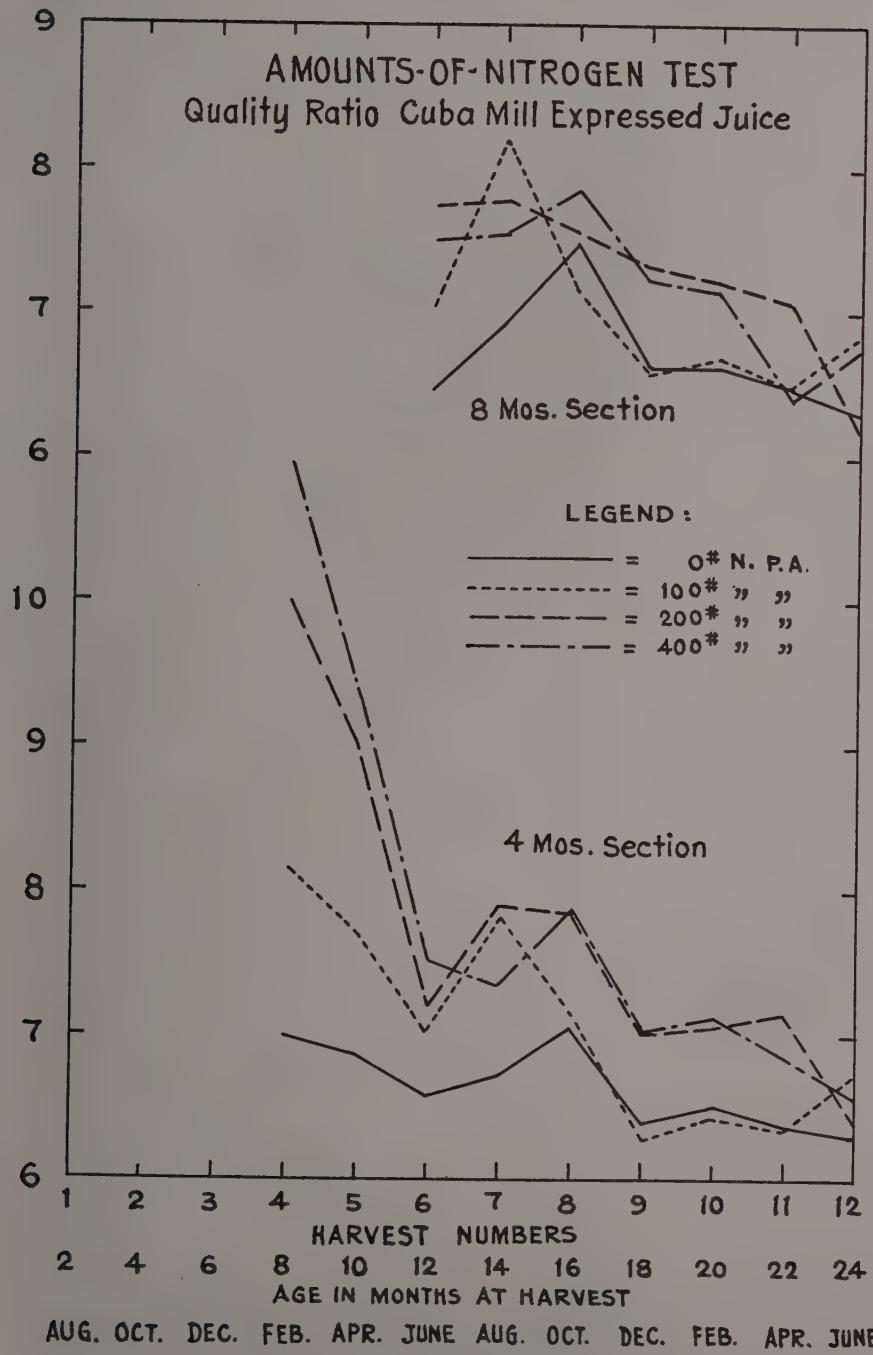


Fig. 9

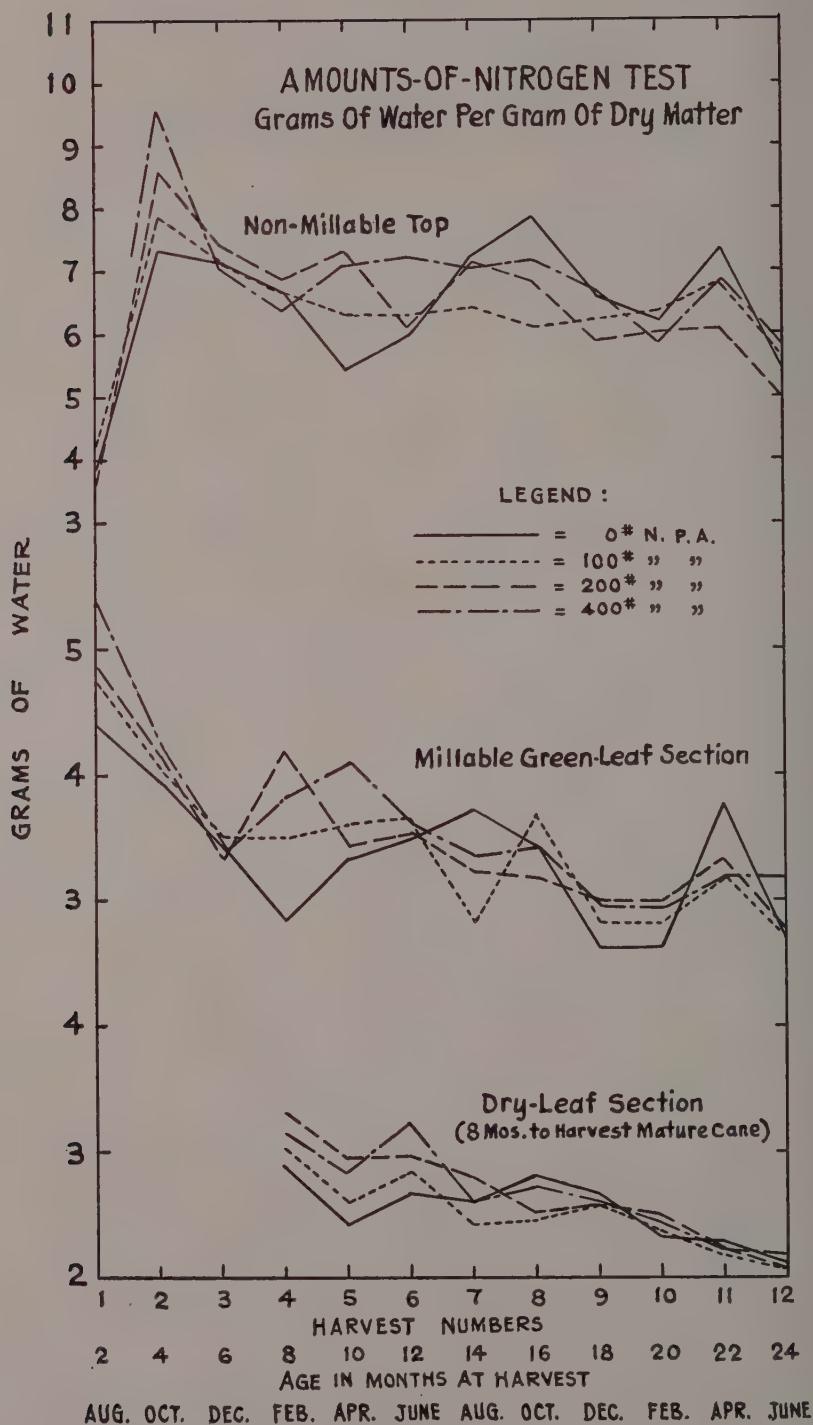


Fig. 10

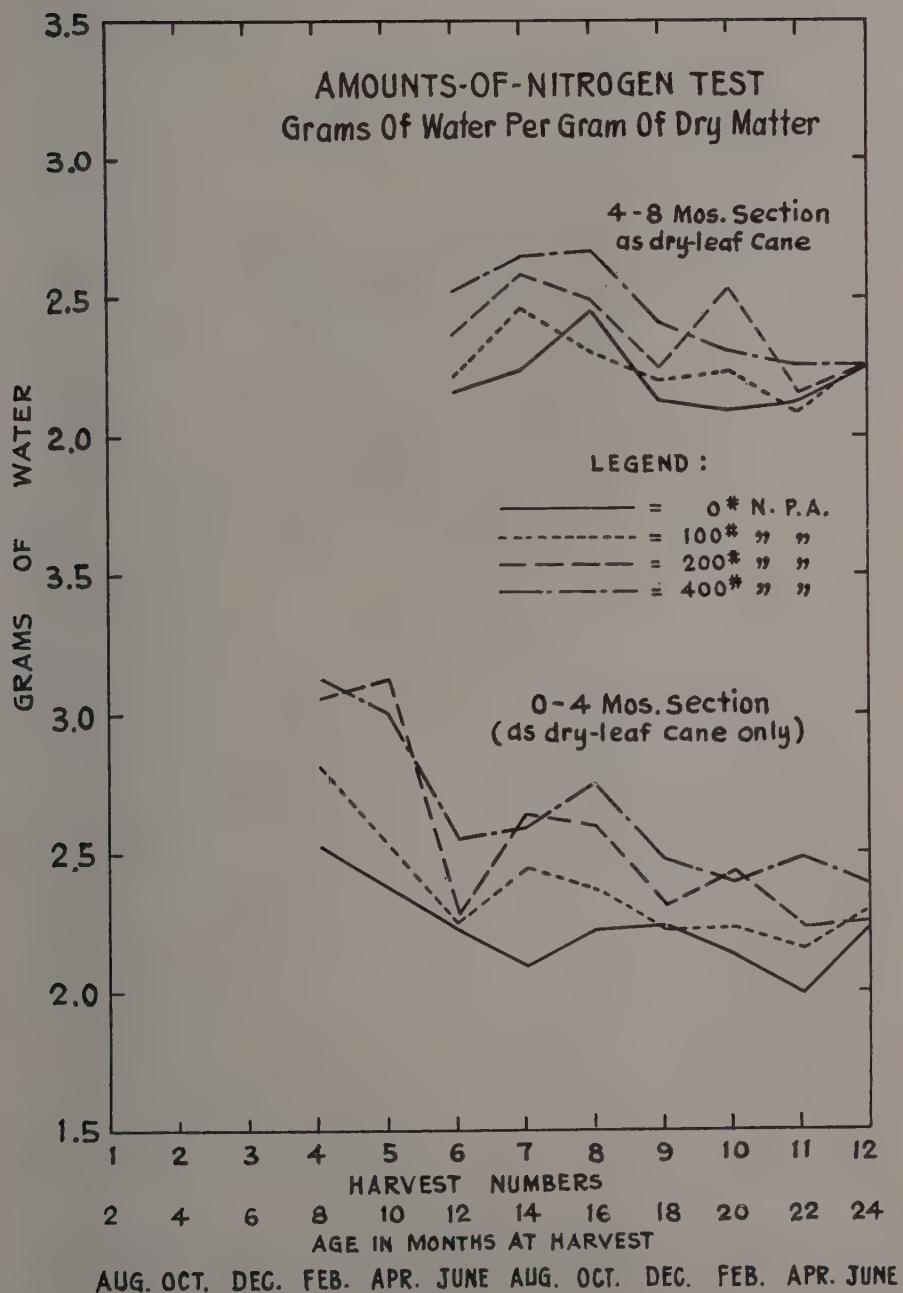


Fig. 11

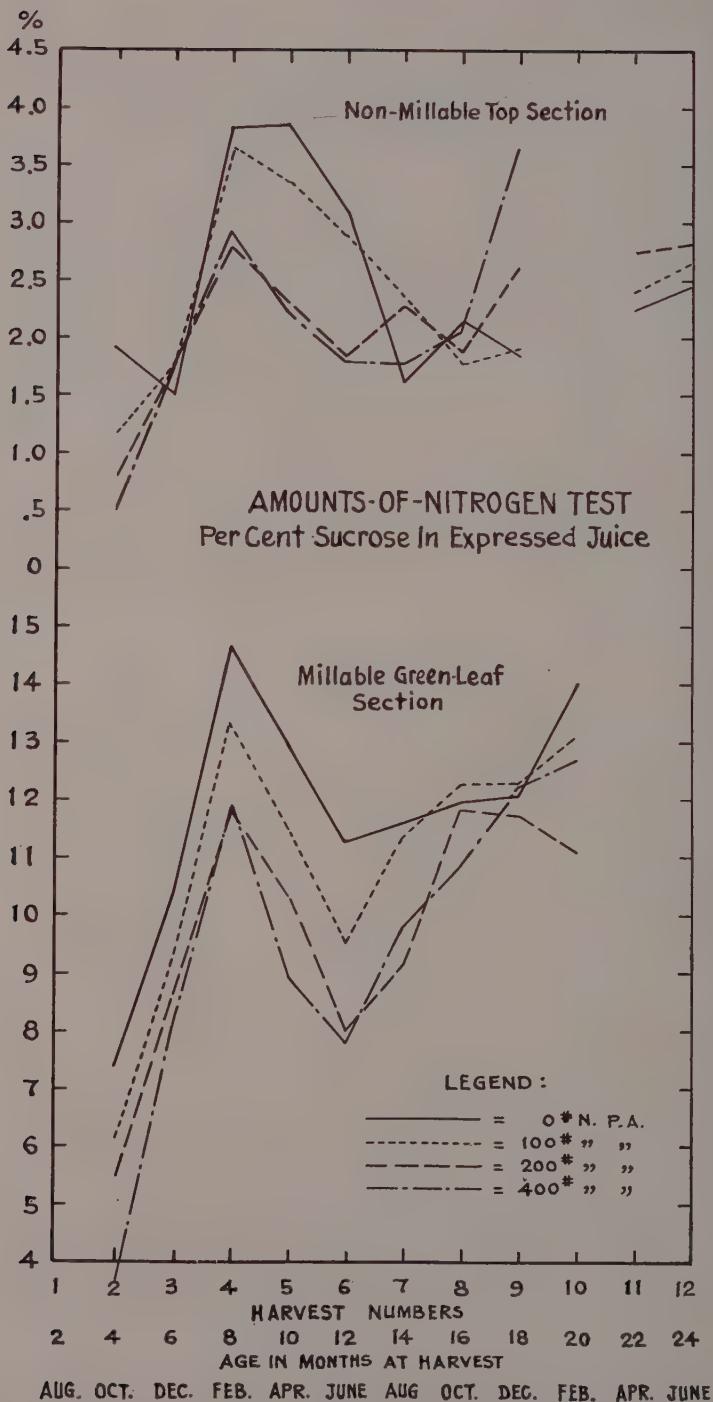


Fig. 12

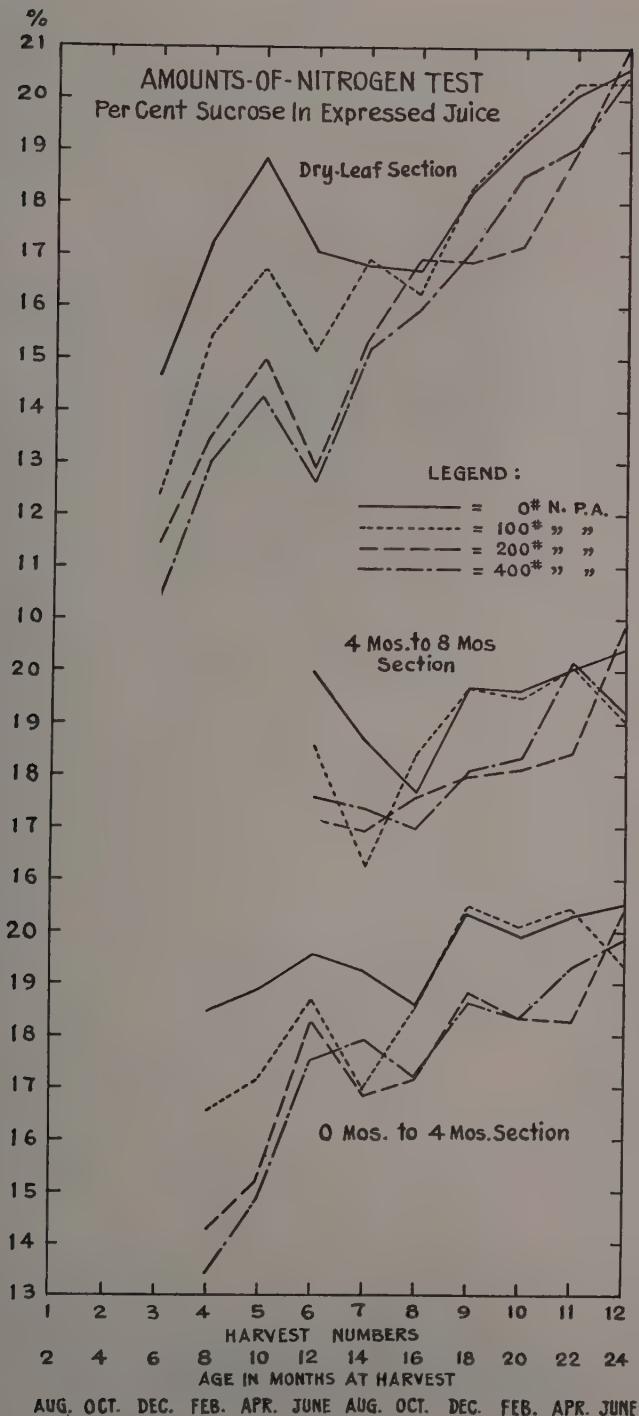


Fig. 13

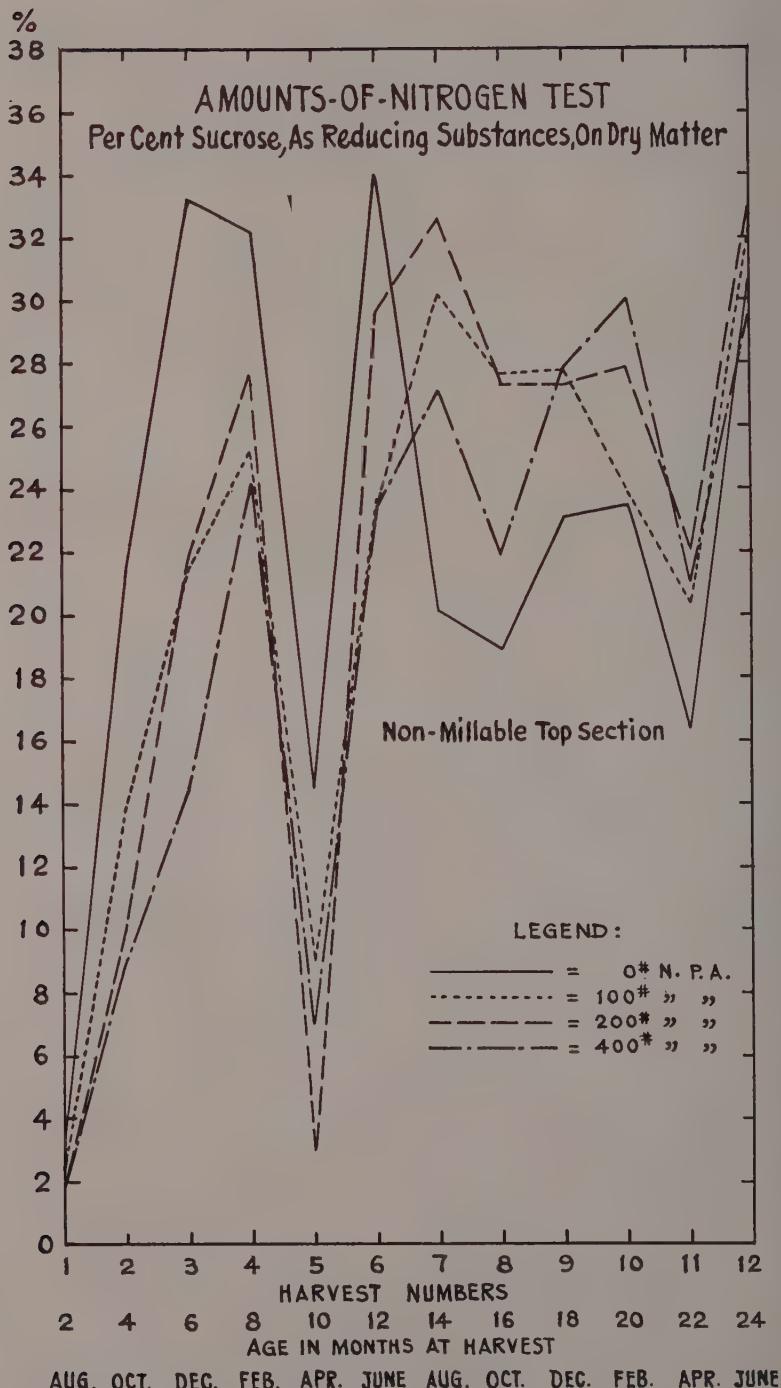


Fig. 14

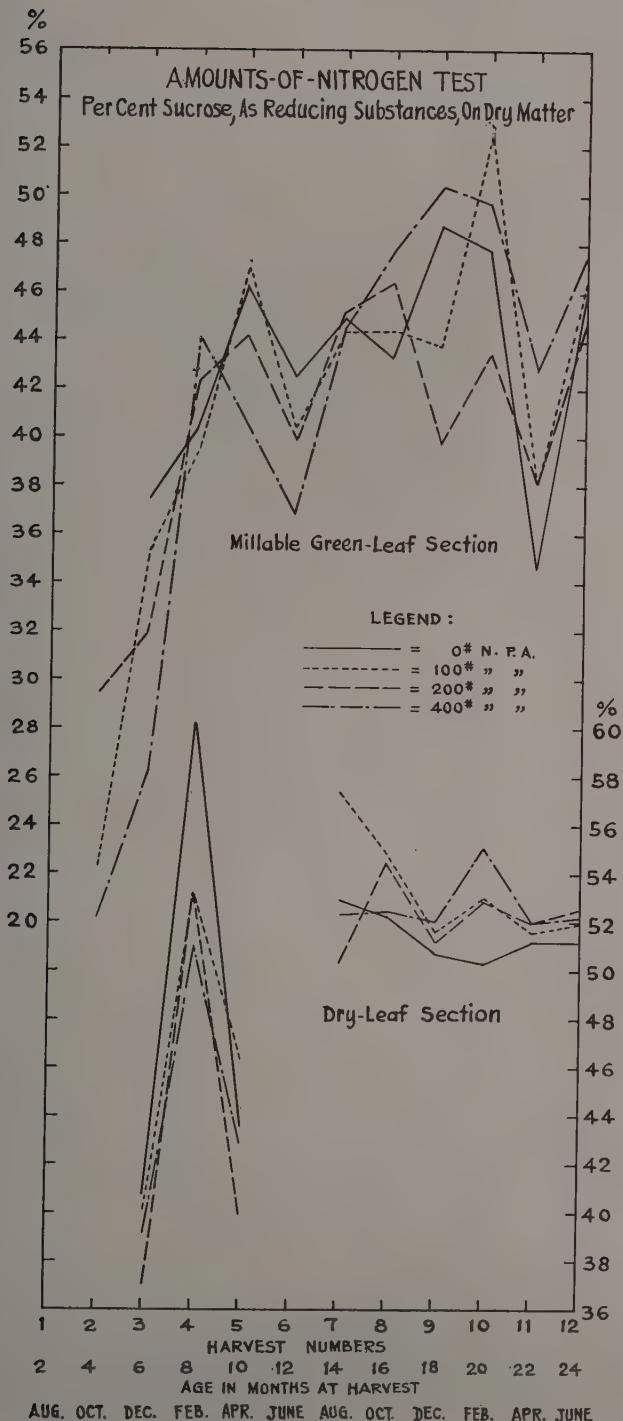


Fig. 15

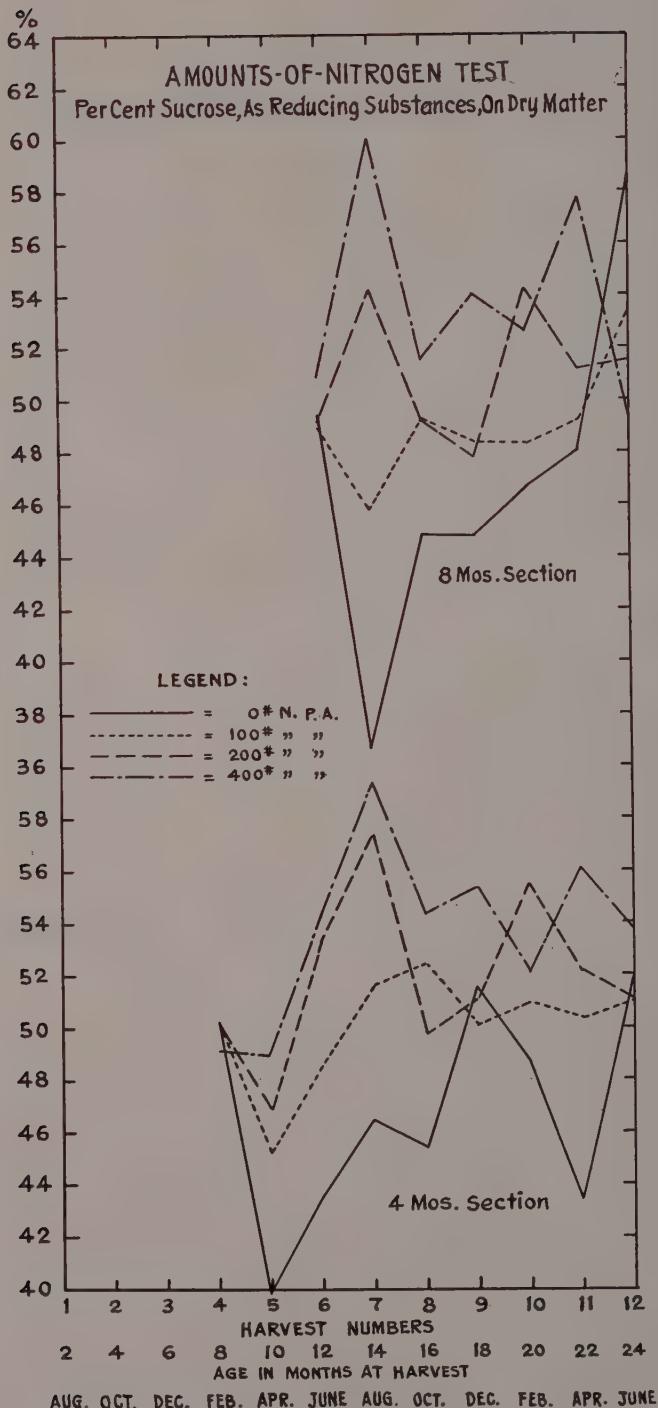


Fig. 16

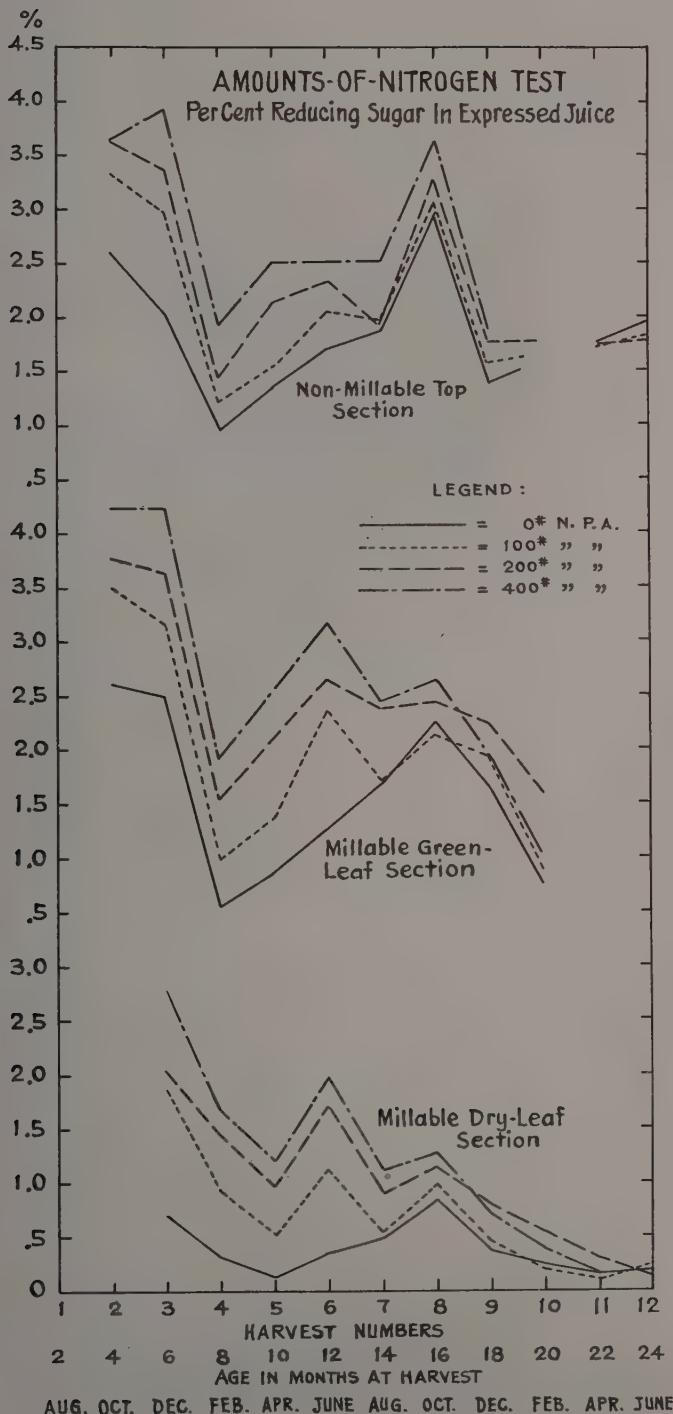
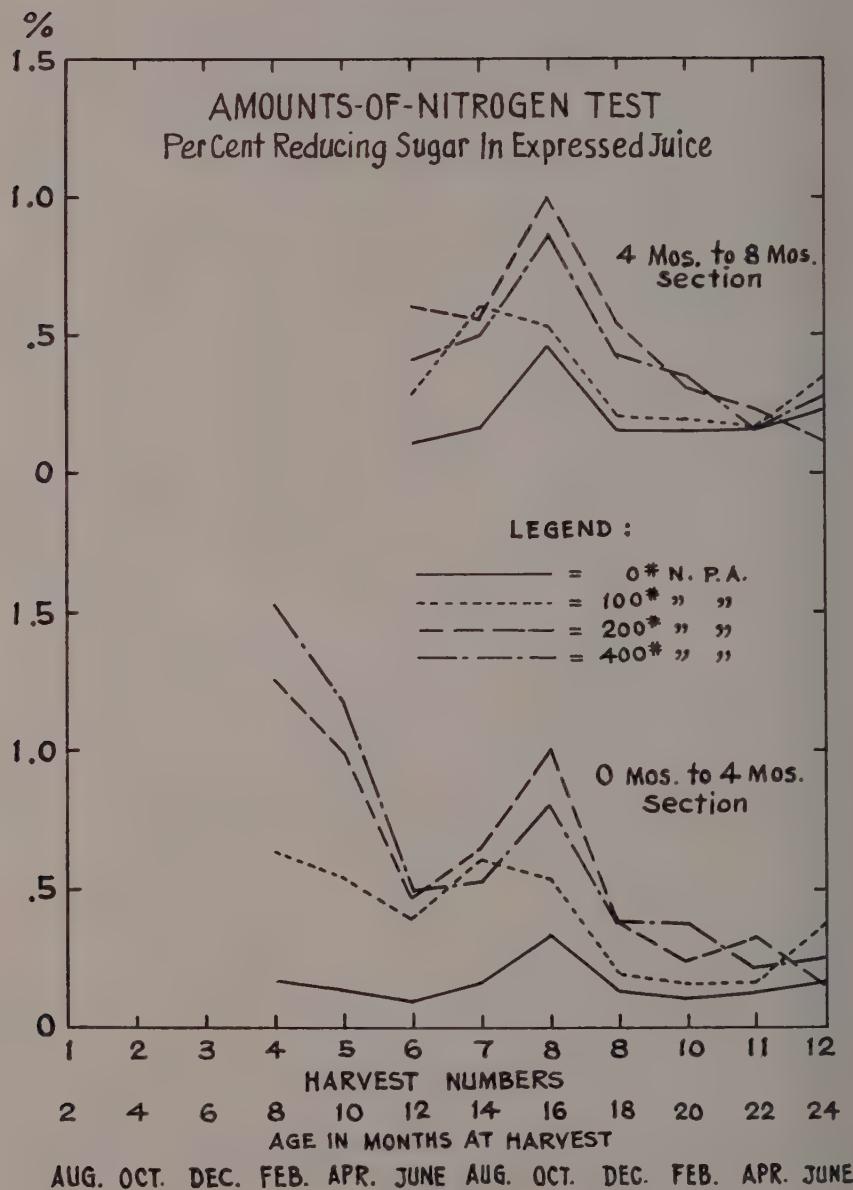


Fig. 17



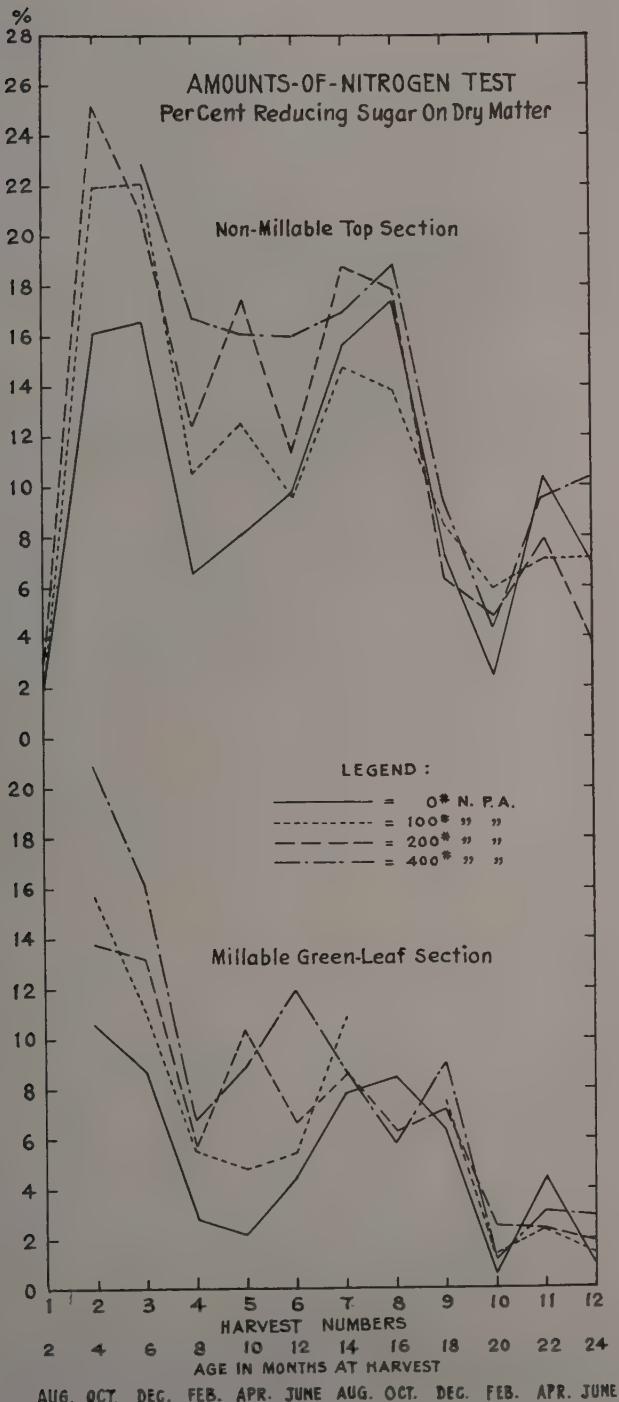


Fig. 19

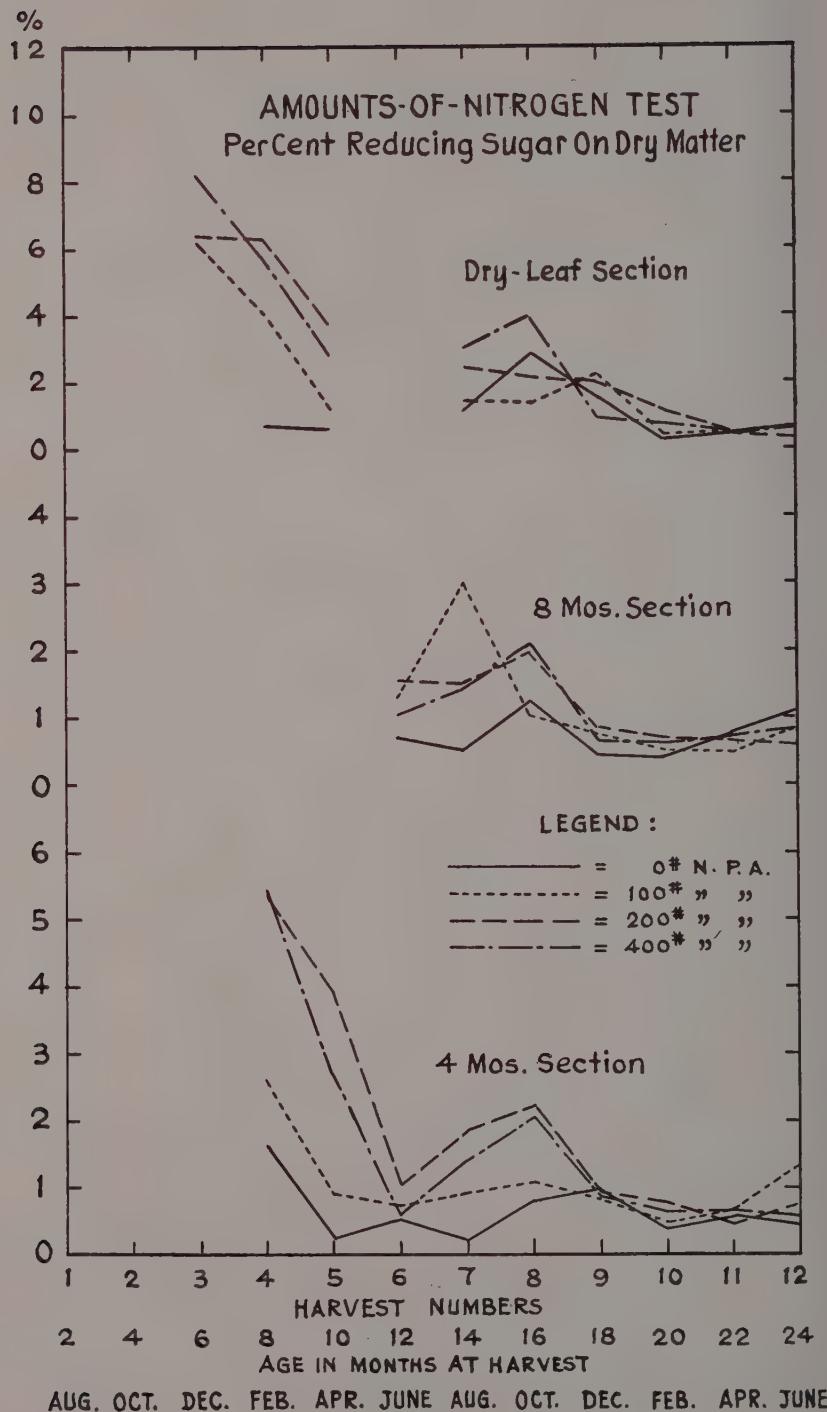


Fig. 20

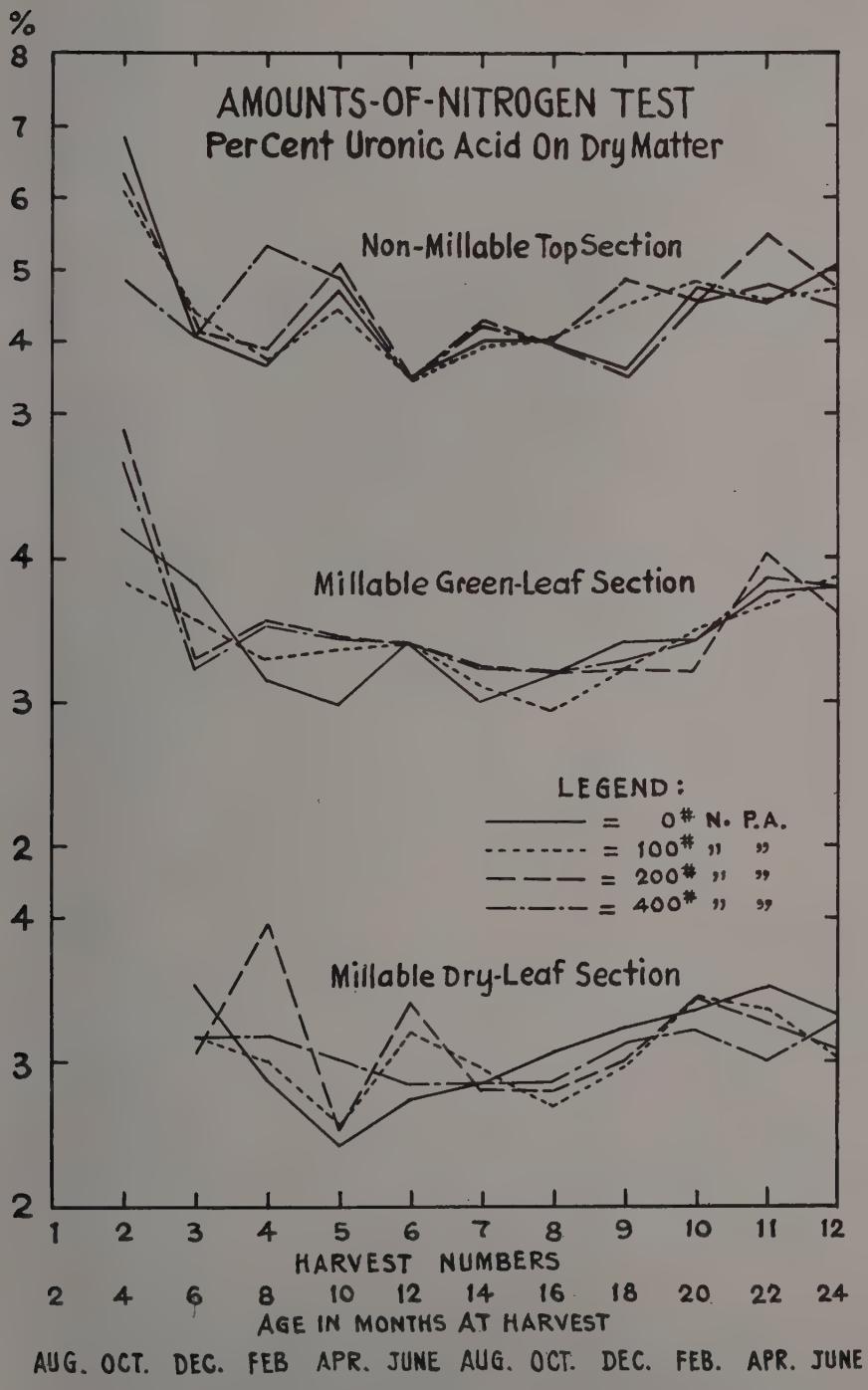


Fig. 21

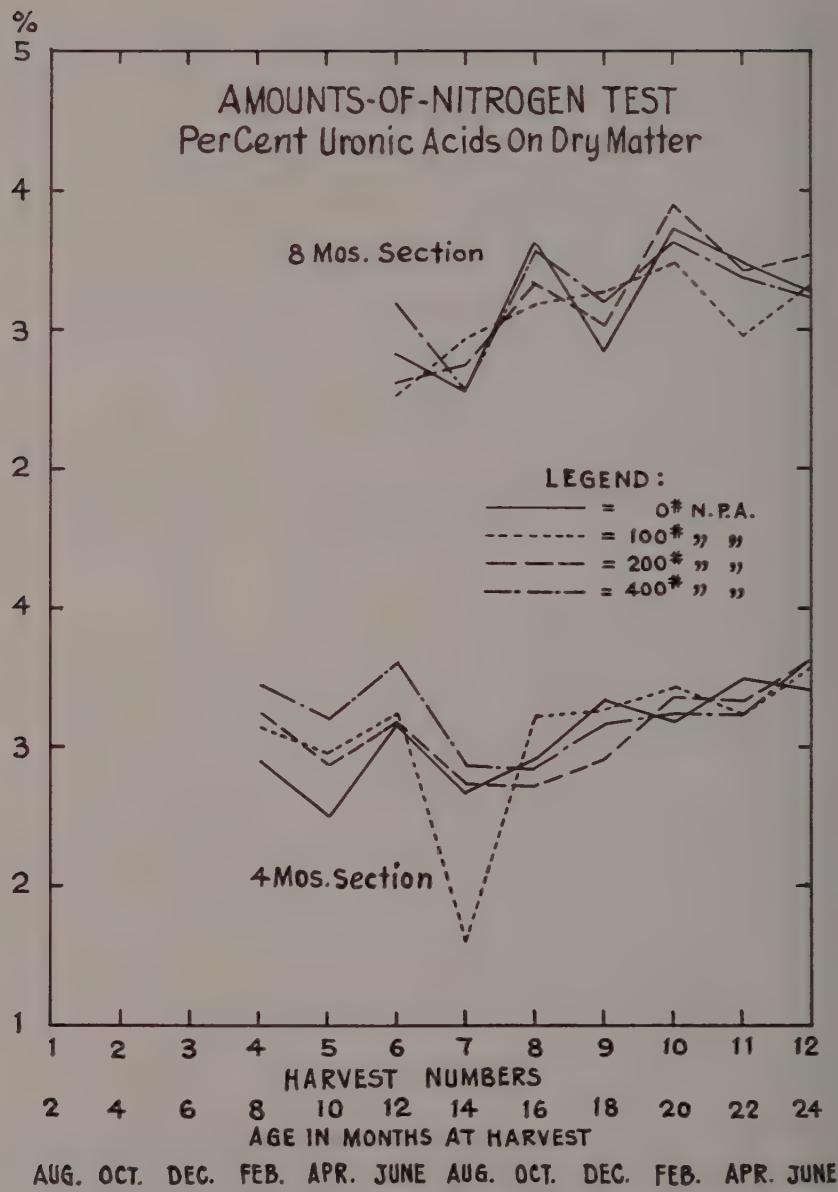


Fig. 22

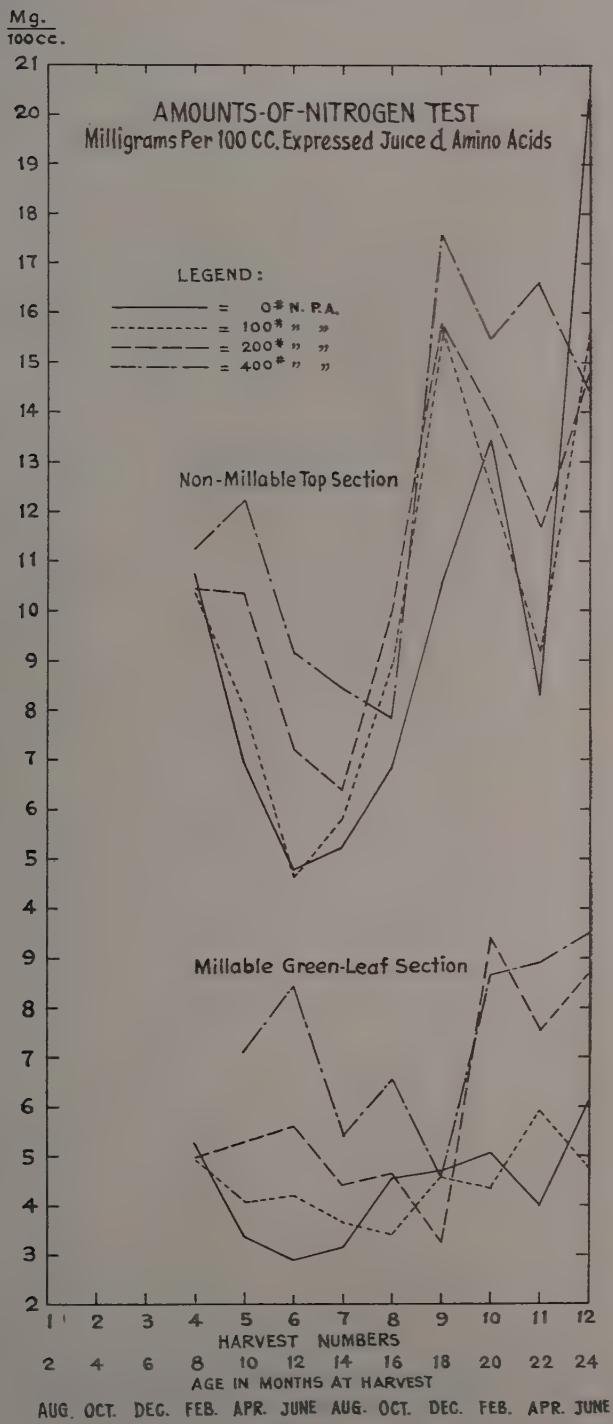


Fig. 23

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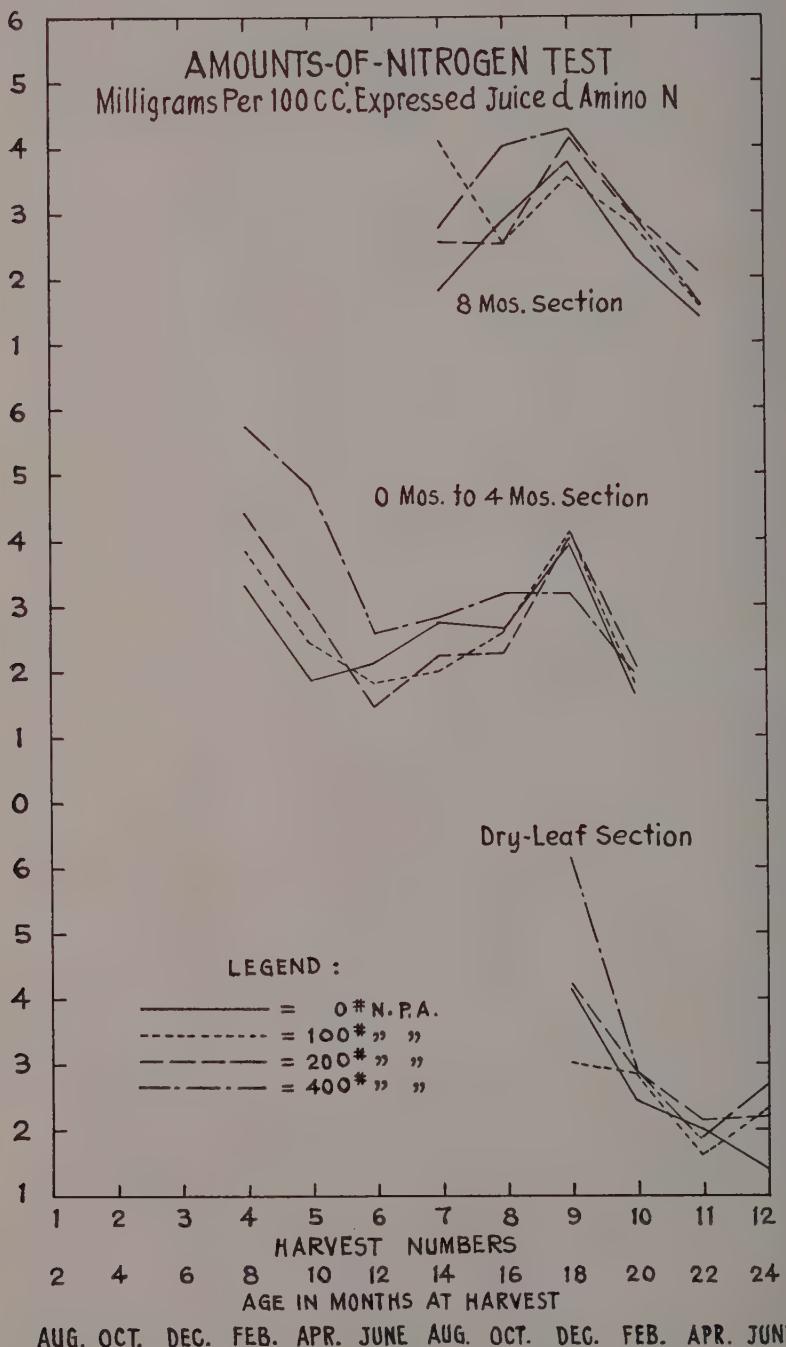


Fig. 24

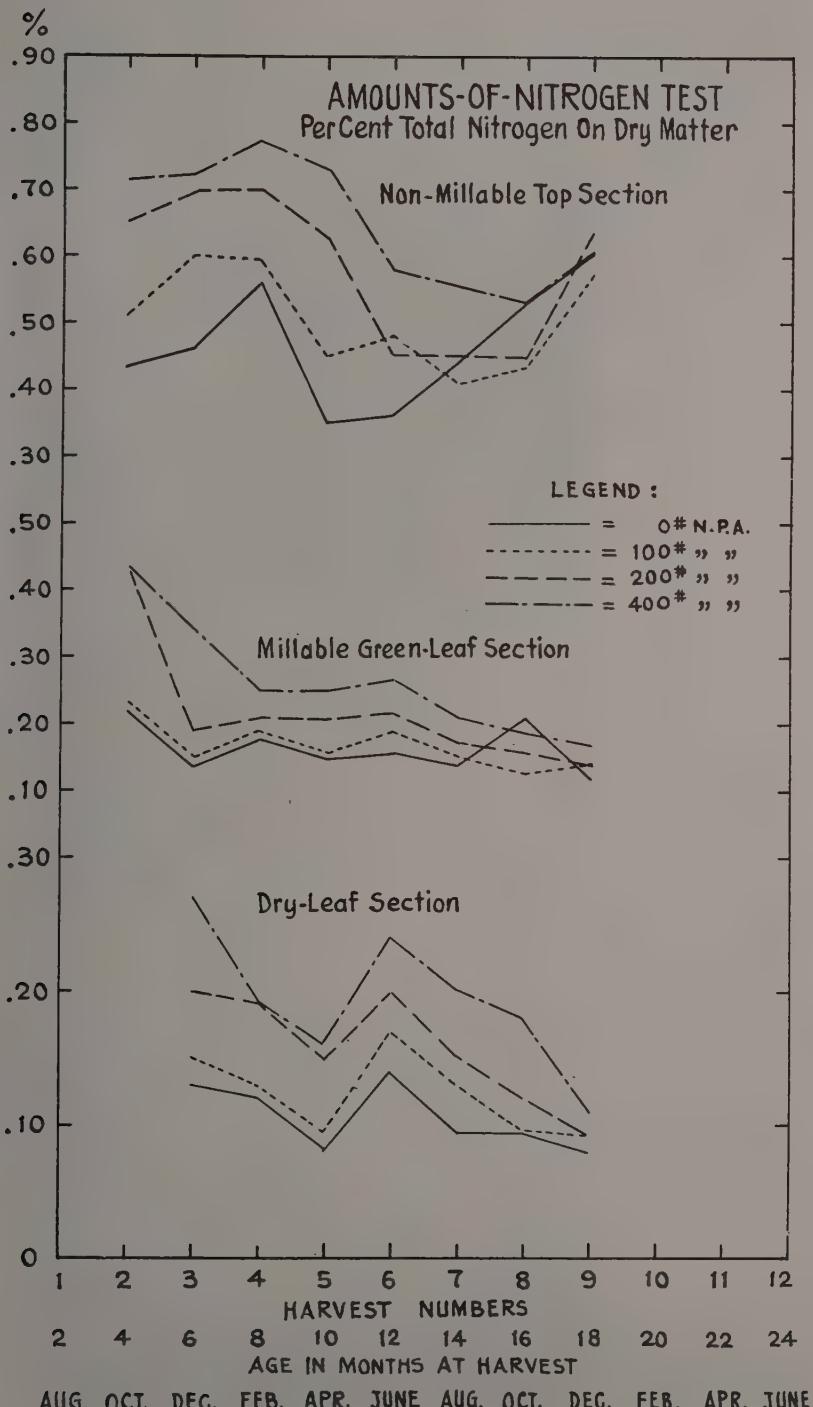


Fig. 25

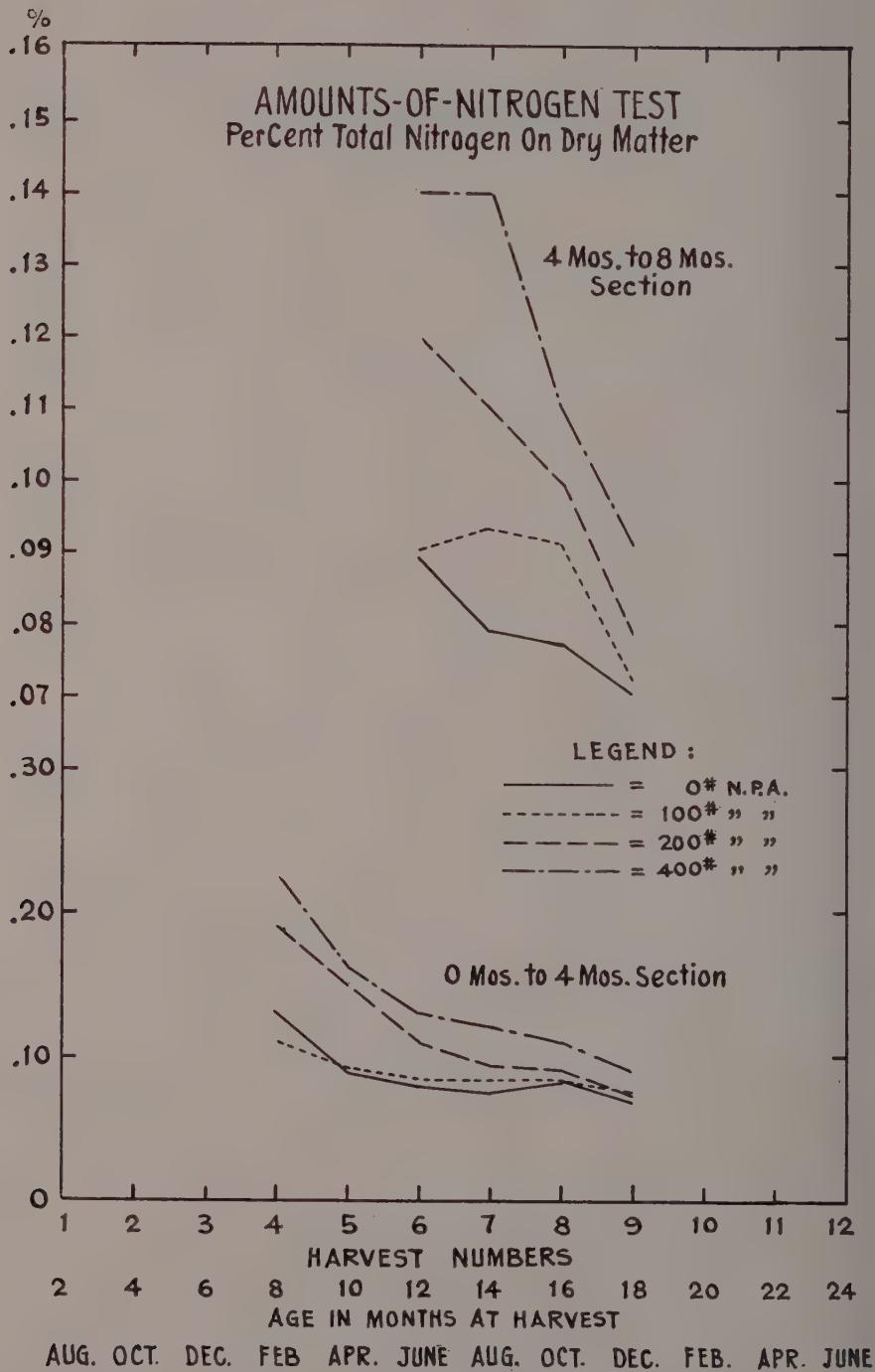


Fig. 26

## Sugar Prices

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### 96° CENTRIFUGALS FOR THE PERIOD

JUNE 21, 1940 TO SEPTEMBER 11, 1940

Date	Per pound	Per ton	Remarks
June 21, 1940.....	2.70¢	\$54.00	Philippines.
" 26.....	2.68	53.60	Philippines.
" 27.....	2.71	54.20	Philippines.
" 28.....	2.75	55.00	Philippines; Puerto Ricos.
July 5.....	2.70	54.00	Philippines; Cubas; Puerto Ricos.
" 16.....	2.68	53.60	Philippines.
" 17.....	2.65	53.00	Philippines.
" 22.....	2.67	53.40	Philippines.
" 26.....	2.63	52.60	Philippines.
Aug. 8.....	2.65	53.00	Philippines.
" 16.....	2.62	52.40	Philippines.
" 27.....	2.70	54.00	Philippines.
Sept. 6.....	2.71	54.20	Philippines.
" 11.....	2.70	54.00	Philippines.

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# THE HAWAIIAN PLANTERS' RECORD

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